FISHES OF THE MIO-PLIOCENE WESTERN SNAKE RIVER PLAIN AND VICINITY

III. INTROGRESSIVE mtDNA TRANSFER IN HYBRID LAKE SUCKERS (TELEOSTEI, CATOSTOMIDAE) IN WESTERN UNITED STATES

by

GERALD R. SMITH, DONALD W. ZAROBAN, BRETT HIGH, JOHN W. SIGLER, J. SCHILLING, TREVOR J. KRABBENHOFT, AND THOMAS E. DOWLING

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Stearley, R. F. and G. R. Smith, 2016. Salmonid fishes from Mio-Pliocene lake sediments in the Western Snake River Plain and the Great Basin. pp. 1-43, 17 figs., 4 tables, 3 maps. *In*: Fishes of the Mio-Pliocene Western Snake River Plain and Vicinity. *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 204.

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COVER PHOTOGRAPH— Specimens from David Cole (figure 3), photographs by Gerald R. Smith.

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ABSTRACT

Hybridization and introgression permitted gene transfer from *Catostomus* to Lake Suckers in modern and MioPliocene lakes of Western United States. Lake Sucker genera, *Chasmistes, Deltistes,* and *Xyrauchen,* were sympatric with species of *Catostomus* (riverine suckers) in four large modern lakes and many fossil lakes in the Great Basin, Klamath, and Columbia-Snake drainages, and also in the Colorado River. Unique morphological traits in Lake Suckers originally included distinctive lips, jaw bones, neurocranial bones, and gill-rakers, but many of the original traits were lost or partly lost, and the remaining phenotypes are mixtures of intermediate morphological traits grading toward local species of *Catostomus*. The intermediates are comparable to patterns in other vertebrate interspecific hybrids. The fossil record documents *Catostomus* and Lake Suckers in many lacustrine localities between the Sierra Nevada and the Great Basin at least 7 million years ago; but mitochondrial DNA of each species of Lake Sucker is now more like its sympatric or nearby *Catostomus* than to other Lake Suckers, indicating mtDNA transfer after or during several million years of evolution. Morphological cladistic, DNA, chromosome, and fossil analyses of Lake Suckers suggest that the mixed-phenotype pattern in Lake Suckers is consistent with hypothesized introgression rather than recent origin of *Chasmistes* phenotypes from sympatric *Catostomus*. Catostomids are tetraploid with duplicate genes at many loci. Duplicate chromosomes and genes may shield these organisms from the effects of hybrid mismatched genomes, but not without dosage distortion and related conflicts, including possible sterility.

Key words: *Chasmistes, Deltistes, Xyrauchen*, *Catostomus*, Utah Lake, Upper Klamath Lake, Pyramid Lake, Jackson Lake, Palisades Reservoir, Mono Lake, Owens Lake, mtDNA, sterility, ecophenotypy, apomorphy loss, tetraploid, fossils

INTRODUCTION

The consequences of introgression for evolutionary processes are controversial. Hybridization may be positive or negative for adaptation and diversification but interbreeding is often rejected by zoologists for philosophical reasons (Hubbs 1955; Arnold, 2016). Extreme examples of introgression were documented in Lake Suckers and their relatives of Upper Klamath Lake, Oregon (Andreasen, 1975; Dowling et al., 2016), and ancient lakes of the Great Basin (Miller and Smith, 1981). Lake Suckers are a clade of catostomid fishes that have been important inhabitants of western U.S. lakes and rivers through the late Miocene, Pliocene, and Pleistocene into modern times. Fossils document about 7 million-years (m.y.) of history of *Chasmistes* and other Lake Suckers, *Deltistes,* and *Xyrauchen*, in the Great Basin, Klamath, Snake-Columbia drainage, and Colorado River. Abundant fossils are found in more than 40 localities in Idaho, Oregon, Washington, Nevada, and California (Fig. 1; Miller and Smith, 1967; Smith, 1975; Kimmel, 1975, 1982; Miller and Smith, 1981; Smith et al.,

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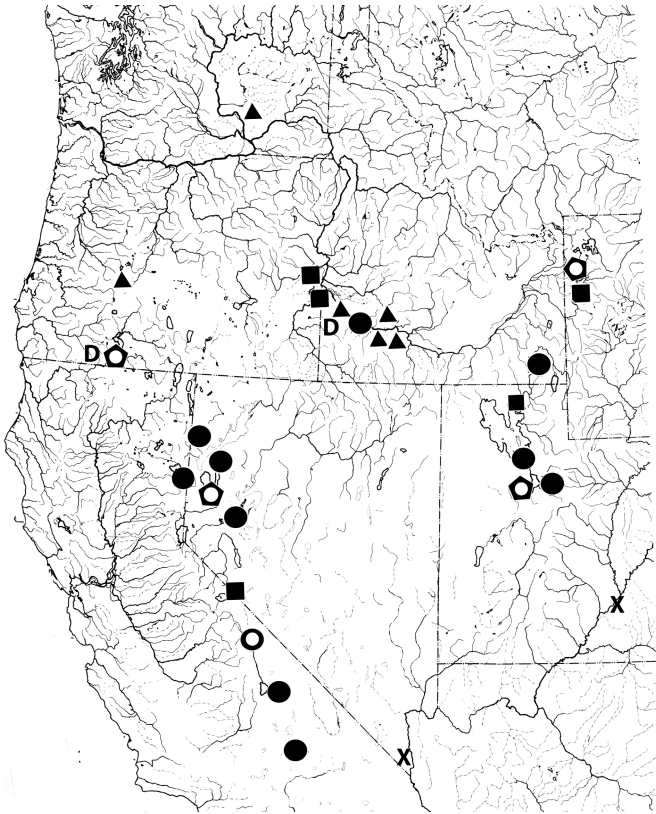
Figure 1.— Localities for Fossil and Extant Lake Suckers in the Northern Great Basin: Upper Klamath Lake, Pyramid Lake, Utah Lake, Jackson Lake, and Palisades Reservoir. Fossil *Chasmistes* are centered in the Mio-Pliocene tectonic trough between the Sierra Nevada and the Great Basin, and the Snake-Columbia drainage. Round symbols, Pleistocene; triangles Pliocene, squares, Miocene. **D**, *Deltistes* in the Klamath drainage and fossil on the Western Snake River Plain; **X**, *Xyrauchen* formerly throughout the Colorado River including Imperial Valley, not shown.
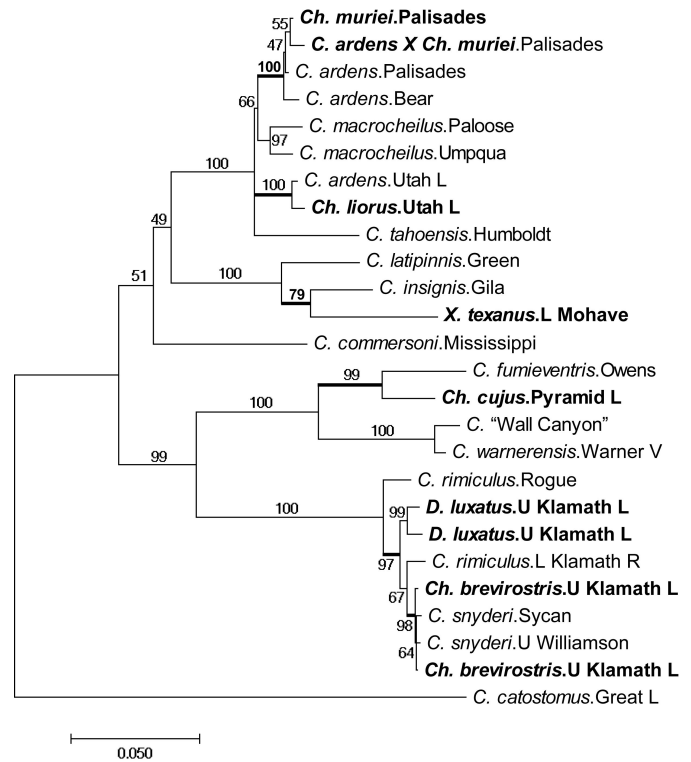

Figure 2.— Maximum likelihood tree showing varying similarity of mtDNA in Lake Sucker, *Chasmistes*, *Deltistes*, and *Xyrauchen*, to mtDNA of sympatric or formerly sympatric *Catostomus*.

1982; Smith et al., 2000; Smith et al., 2002). Lake Suckers are large (~1 kg), planktivorous fishes, which along with their mostly riverine sister genus, *Catostomus*, are important in food webs of aquatic ecosystems in large lakes. Their DNA sequences and inferred phylogenies based on them (Fig. 2) have raised questions about the relationships between DNA, morphology, and evolution.

Chasmistes is diagnosed by a subterminal mouth, plicate rather than papillose lips (Figs. 3, 4), and short, broad neurocrania. *Chasmistes* and other members of the Lake Sucker clade possess a gap between the lower lobes of the lips, more numerous and more branched gill rakers (Figs. 5, 6), elongate jaw bones (Figs. 7, 8), and a sharp keel on the parasphenoid and basioccipital cranial bones (Smith 1992a). *Xyrauchen* of the Pliocene and recent Colorado River and *Deltistes* of the Mio-Pliocene Western Snake River Plain and modern Klamath drainage (Fig. 1) are less advanced members of the clade, with ventral mouths, papillose lips (Fig. 3) and

long, narrow neurocrania. *Chasmistes* species were formerly highly-derived members of the Lake Sucker clade. *Chasmistes* collected in the Nineteenth and early Twentieth Century are diagnosed by their advanced traits including the short, broad dermethmoid and wide anterior half of the skull (Figs. 9, 10; Smith, 1975; 1992a), but these traits are being lost in all but Pyramid Lake over time, as habitats in lakes and rivers are impacted by human pollution and water withdrawal.

Growth, feeding ecology, and reproductive ecology of modern Lake Suckers are being studied by ecologists and conservation biologists, especially at Pyramid Lake, Nevada, Upper Klamath Lake, Oregon, and Utah Lake, Utah (Belk, 1998; Ellsworth et al., 2010; Belk and Schaalje, 2016; Scoppettone and Vinyard, 1991; Cooke et al., 2005; Terwilliger et al., 2010; USFWS, 2012). Neutral DNA sequences of Lake Suckers and related sympatric species of *Catostomus* (Fig. 2) have been documented by several laboratories (Dowling et al., 2005, 2014; Tranah and May, 2006; Mock et al., 2006; Doosey et al., 2010; Chen and Mayden, 2012; Dowling et al., 2016). Mitochondrial DNA (mtDNA) phylogenies have identified variation in gene sequences that are discordant relative to morphological studies (Mock et al., 2006; Doosey et al., 2010; Cole, 2014; Dowling et al., 2016; Bagley et al., 2018). The initial hypothesis to explain discordant patterns of mitochondrial and other DNA and other neutral molecular

a

c

b

d
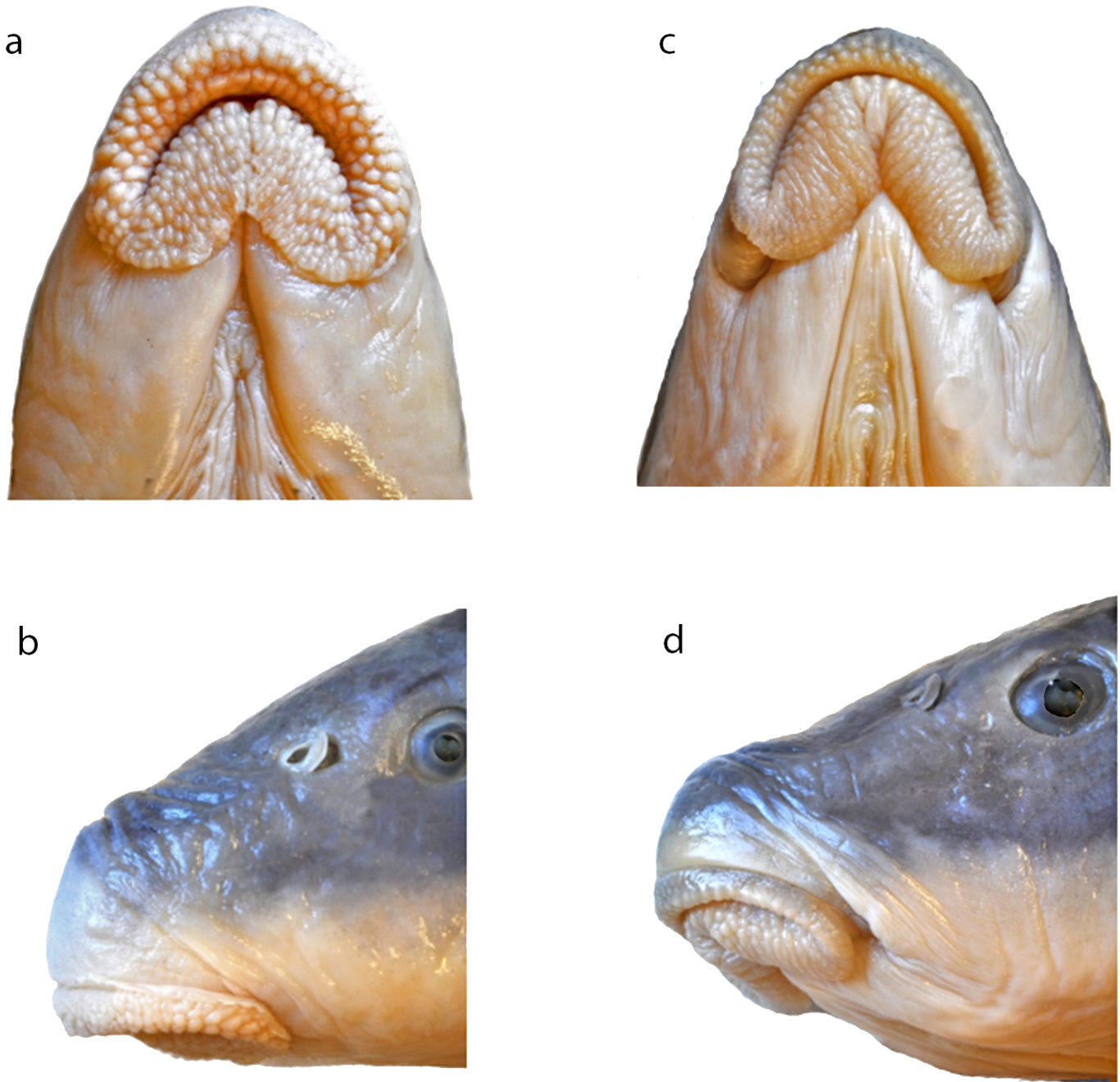
Figure 3.— Lips, mouths and profiles of Jackson Lake Suckers resembling *Catostomus ardens*, **a**, **b**, and hybrid *Catostomus ardens* × *Chasmistes muriei*, **c**, **d**, with diluted *Chasmistes* lips and profile.

markers (Tranah and May, 2006; Dowling et al., 2016) with morphological variation is introgressive hybridization and transfer of mtDNA from *Catostomus* into Lake Suckers. But hybridization, with asymmetric replacement of mtDNA (Ferris et al., 1983) and other neutral genes into Lake Suckers, is not understood and requires examination of evolutionary history,

ecology, and genetics. All populations of the Lake Sucker clade, including *Xyrauchen*, which now lives in rivers and reservoirs in the Colorado River drainage, have a documented record of hybridization and introgression with sympatric species of river suckers, *Catostomus* (Figs. 2, 11c; Miller and Smith, 1981; Smith, 1992a, b). Hybridization was confirmed

Figure 4.— Lips of Palisades suckers; **a-f**, showing weak plicae replacing papillae, stronger gap between lower lips; **d**, and evidence for hybridization, seen in random asymmetry in Palisades suckers; two resemble Utah Suckers, **g**, **h**.
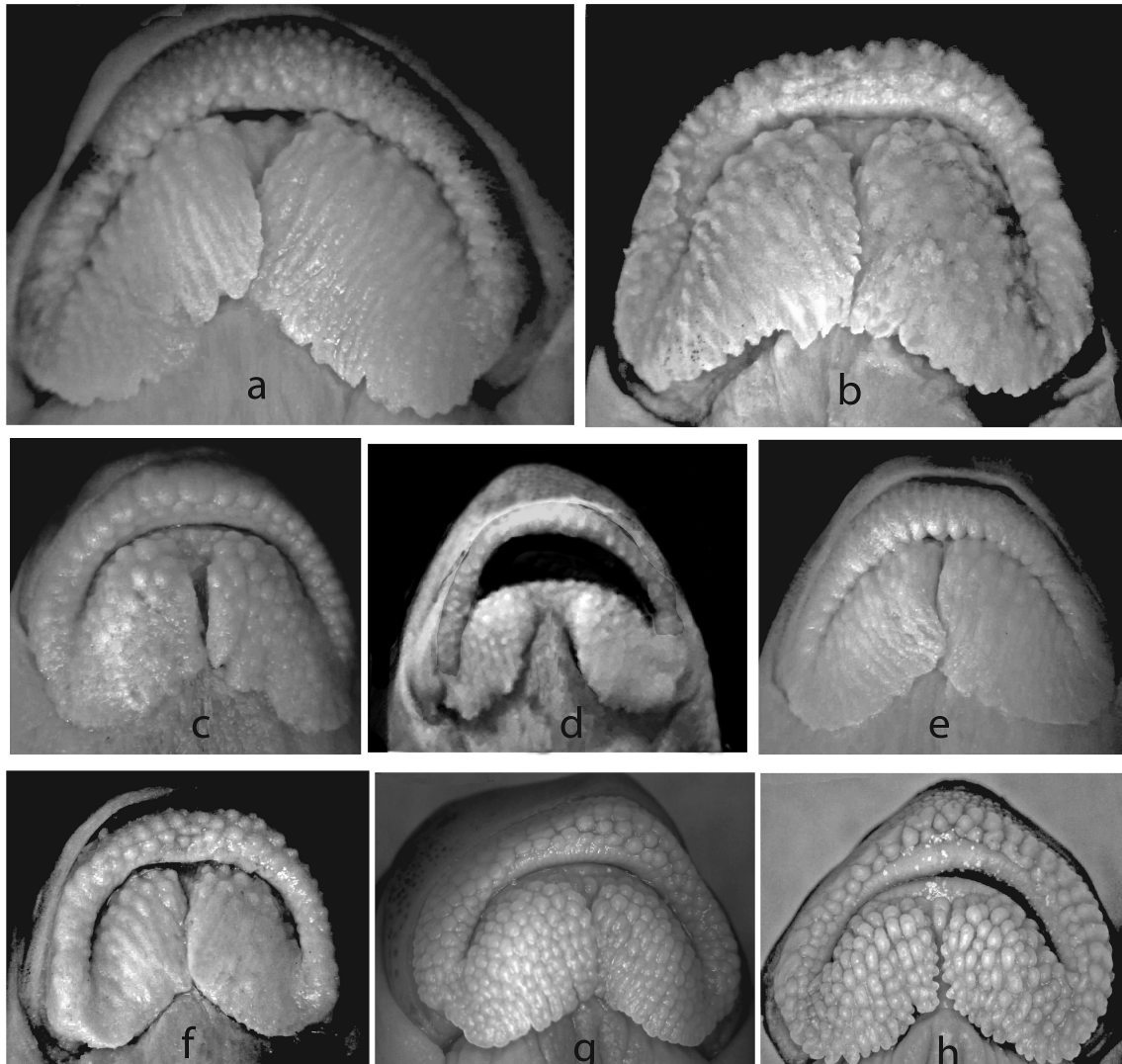
by analyses of mitochondrial DNA and nuclear DNA markers (Dowling et al., 2016), which demonstrated *Catostomus* mtDNA in fishes with Lake Sucker traits.

Hybridization is a controversial phenomenon. It has been abundantly documented but requires detailed study when it involves lateral transfer of mtDNA between lineages with millions of years of divergence, like catostomids. All catostomids are tetraploids with 100 chromosomes. Therefore, crosses between *Catostomus* and Lake Suckers might be expected to exhibit distorted segregation, epigenetic remodeling, and enhancement or repression of some gene functions. The universal transfer of mtDNA between *Catostomus* and Lake Suckers, with complete loss of Lake Sucker mtDNA and its replacement with introgressed sympatric *Catostomus* mtDNA (Fig. 2) is a poorly-understood problem.

The ecology, historical circumstances, and genetic mechanisms by which mtDNA of all members of a clade could have been replaced by introgression from sympatric relatives has been questioned. Chen and Mayden (2012) argued against hybridization and noted that the genetic basis of morphological traits is not known, despite genetic evidence. Various molecular and morphological studies of the Utah Lake complex of *Ch. liorus* and *C. ardens* (Mock et al., 2006, Cole et al., 2008) led authors to conclude that these species are recently diverging, with their morphological distinctiveness resulting from the action of strong ecological selection, as opposed to recent loss of divergence caused by introgressive hybridization. In his study of *Ch. muriei − C. ardens* complex in Utah Lake and Jackson Lake, Cole (2014) found similar shallow molecular divergences coupled with few observed external discriminating traits and concluded that
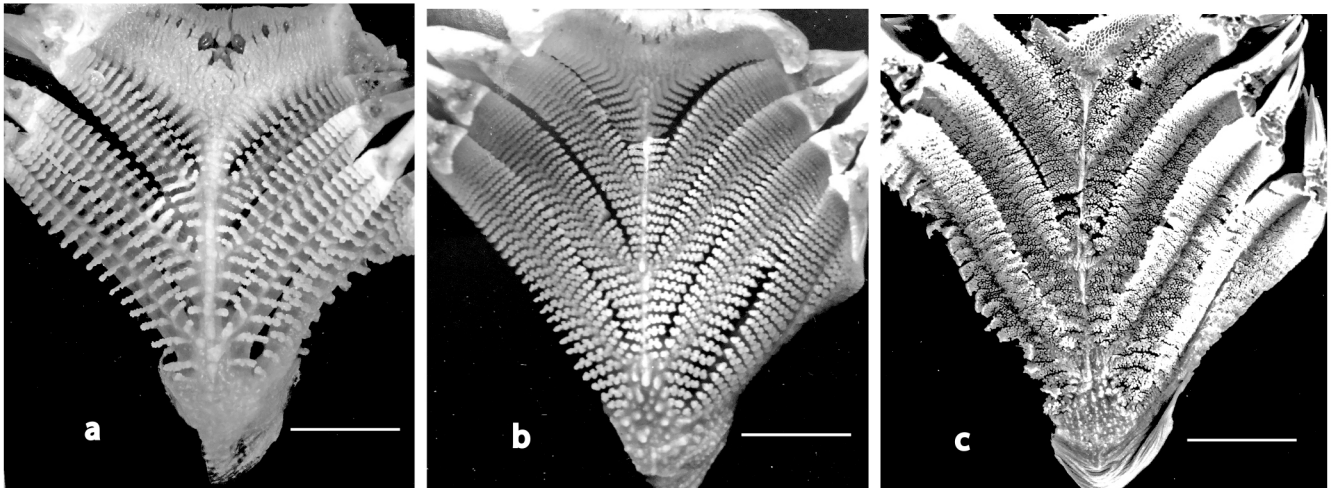
Figure 5.— Branchial baskets of **a**, *Catostomus latipinnis*; **b**, *Xyrauchen*; and **c**, *Chasmistes brevirostris* (from Willink, 2002) showing progressive branching density reaching its peak in *Chasmistes*, adapted to feeding on plankton.

the minor morphological differences likely reflected the action of recent ecological selection within populations of *C. ardens*. This perspective on shallow divergence has been extended to include other species of *Chasmistes* in Pyramid and Klamath lakes (implied by Mock et al., 2006; Cole, 2014; Harris et al., 2014). This limited divergence and non-monophyly of mtDNA led to rejection of hybridization as an explanation and denial of existence of *Chasmistes* as a taxon (Chen and Mayden, 2012). But none of these authors studied the previously-documented osteological differences or the fossil record.

The alternative interpretations of the discordance of morphological and mtDNA data require a more complete discussion of the broader Lake Sucker clade, its geological history, and its universal proclivity to hybridize with and incorporate genes from many sympatric relatives. The purpose of this study is to investigate morphological and mtDNA data of Lake Suckers (*Chasmistes, Deltistes, and Xyrauchen*) and sympatric river suckers (*Catostomus*). If two unhybridized species were present in a sample, we expect to see bimodal character distributions correlated among the several mouth, gill raker, and osteological differences. If backcross or F$_1$ hybrids are present we expected to see intermediates in scatter plots between parental types, grading toward one or both parents; if generations of backcrossing have occurred we expect to see parental morphological traits variable, uncorrelated, and scattered by mixing of diverged polyploids, with possible loss of *Chasmistes, Deltistes, and Xyrauchen* mtDNA (Fig. 11; Dowling et al., 2005; Dowling et al., 2016; Unmack et al., 2014).

If, on the other hand, Lake Suckers were simply locally- and recently-evolved forms of sympatric *Catostomus*, we expect to see no evidence of hybridization and no significant mtDNA divergence between Lake Suckers and their sympatric *Catostomus* species. Lake Suckers should have pure, modern *Catostomus* mtDNA and little evidence of ancient genetic, morphological, or paleontological differentiation.

Here we test the hypothesis that hybridization occurred among *Deltistes luxatus, Chasmistes brevirostris, Catostomus snyderi,* and *Catostomus rimiculus* in the Klamath Basin, Oregon and California (Dowling et al., 2016); between *Chasmistes cujus* and *Catostomus tahoensis* of Pyramid Lake, Nevada, as well as *Catostomus fumeiventris* of Owens Lake, California; between *Chasmistes liorus* and *Catostomus ardens* of Utah Lake, Utah; and between *Chasmistes muriei* and *Catostomus ardens* in Jackson Lake, Wyoming (Mock et al., 2006; Cole et al., 2008); and between *Xyrauchen texanus* and *Catostomus insignis* and *C. latipinnis* in the Colorado River (Hubbs and Miller, 1953; Buth et al., 1987). The analyses include a test of ongoing introgressive mixing of *Chasmistes* with *Catostomus* morphological and mtDNA traits in Palisades Reservoir, Idaho. The Palisades Reservoir populations also permit investigation of possible male sterility in hybrids.

Hybridization among Catostomid Fishes.— Carl Hubbs and colleagues (Hubbs et al., 1943; Hubbs and Hubbs, 1947; Hubbs and Miller, 1953; Hubbs, 1955) recognized inter-generic hybrids among many combinations of western U. S. suckers in their field work in the 1930s and 1940s. These hybrids were easy to identify because lip and mouth shapes between *Catostomus* and other genera are so different that intermediate specimens are salient (Fig. 11). Intermediates were sometimes named as new species by Nineteenth and early Twentieth Century workers (Hubbs and Bailey, 1952); comparison with larger samples demonstrated that *Catostomus fecundus* Cope (Utah Lake) and *Chasmistes stomius* Gilbert (Upper Klamath Lake) are intermediate *Chasmistes* x *Catostomus* hybrids (Miller and Smith, 1981). In some non-introgressed samples, suspected backcross individuals were shown to be interjacent and graded toward *Catostomus* rather than intermediate
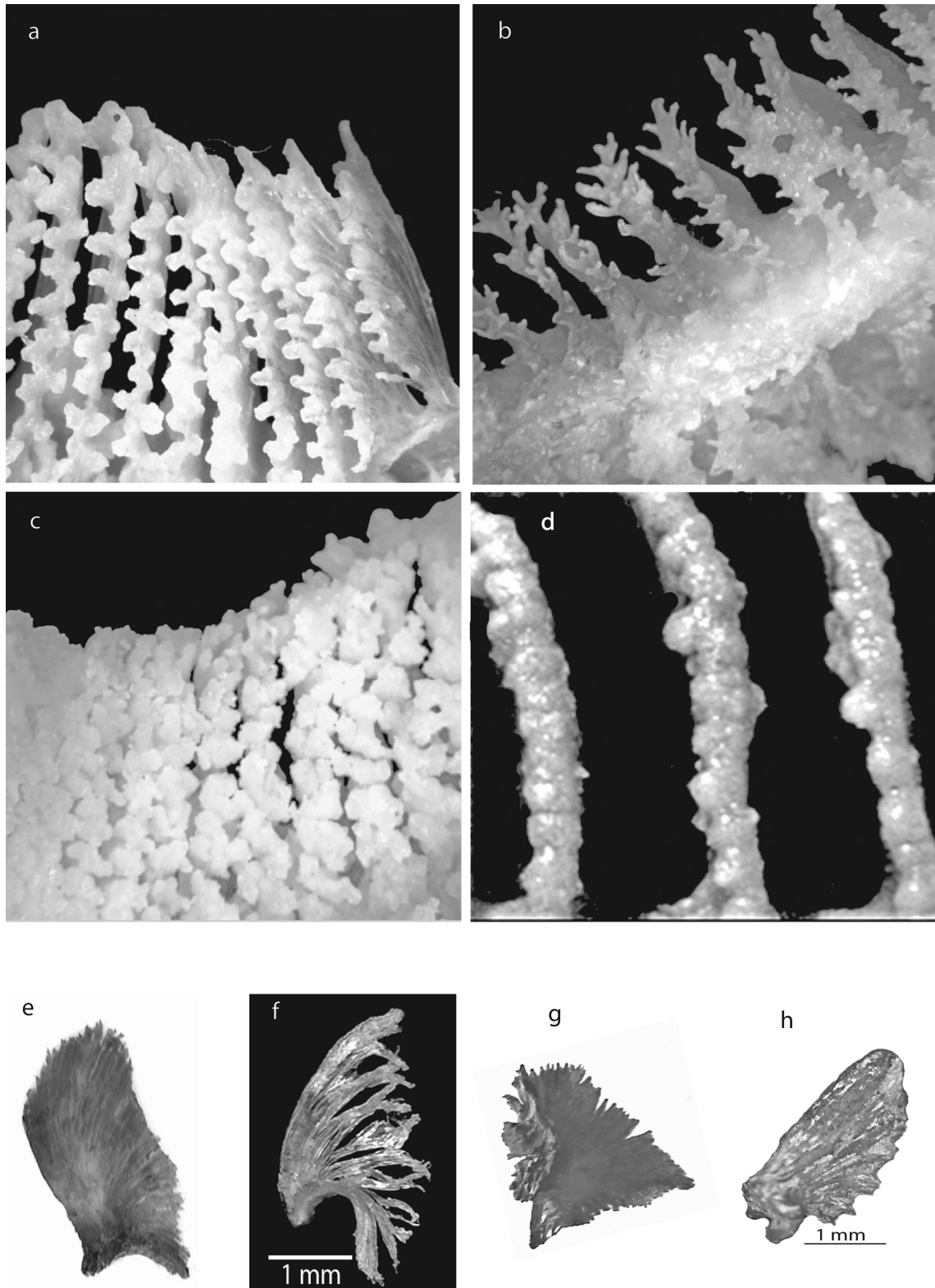
Figure 6.— **a-c**, Branched gill rakers of hybrid *Catostomus* × *Chasmistes* in Palisades Reservoir; **d**, unbranched gill rakers of *Catostomus ardens*. Branched gill rakers never occur in *Catostomus* (Miller and Smith 1981; 5D, B, C). Dried gill raker bone of **e**, *Ch. fumeiventris*, UMMZ 181667; **f**, cleared and stained hybrid from Palisades reservoir; **g**, Ch. cujus, UMMZ 133839; **h**, *Chasmistes cujus* gill-raker bone collected by Ken Gobalet from Miocene sediments of Mono Lake (Smith et al., 2002).
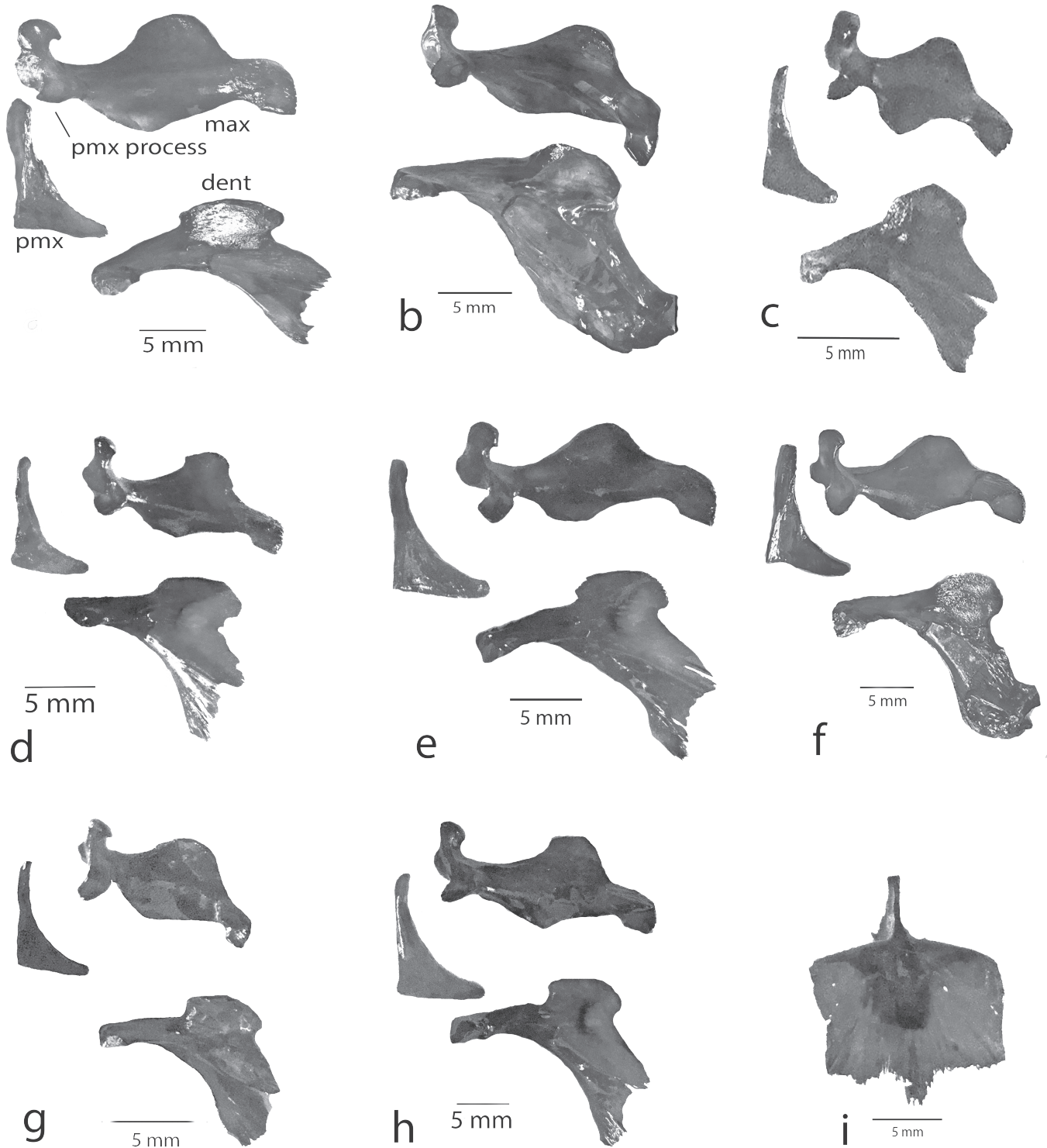
Figure 7.— Jaw bone sets—maxillae, premaxillae, and dentaries—of *Ch. muriei*; **a**, and Palisades suckers, anterior facing left. Angular articular and retroarticular removed in all dentaries except **b** and **i**. Specimens **f**, **g**, and **h**, were identified as similar to Utah Suckers in the field, but gill rakers and jaws indicate introgressed origin of these specimens. Bones of specimens a, b, and e are most like *Chasmistes* in the length of the anterior process of the dentary, the long maxilla, and the reduced ventral keel on the maxilla. Dentaries and maxillae of **c** and **g** are short and shaped like Utah Sucker bones (compare Figure 8; figs. 10 and 11 in Miller and Smith, 1981). Species traits are not correlated within or among specimens in Palisades Reservoir, see Figs. 7a, b, and Table 4. Specimen field numbers are in Table 2.
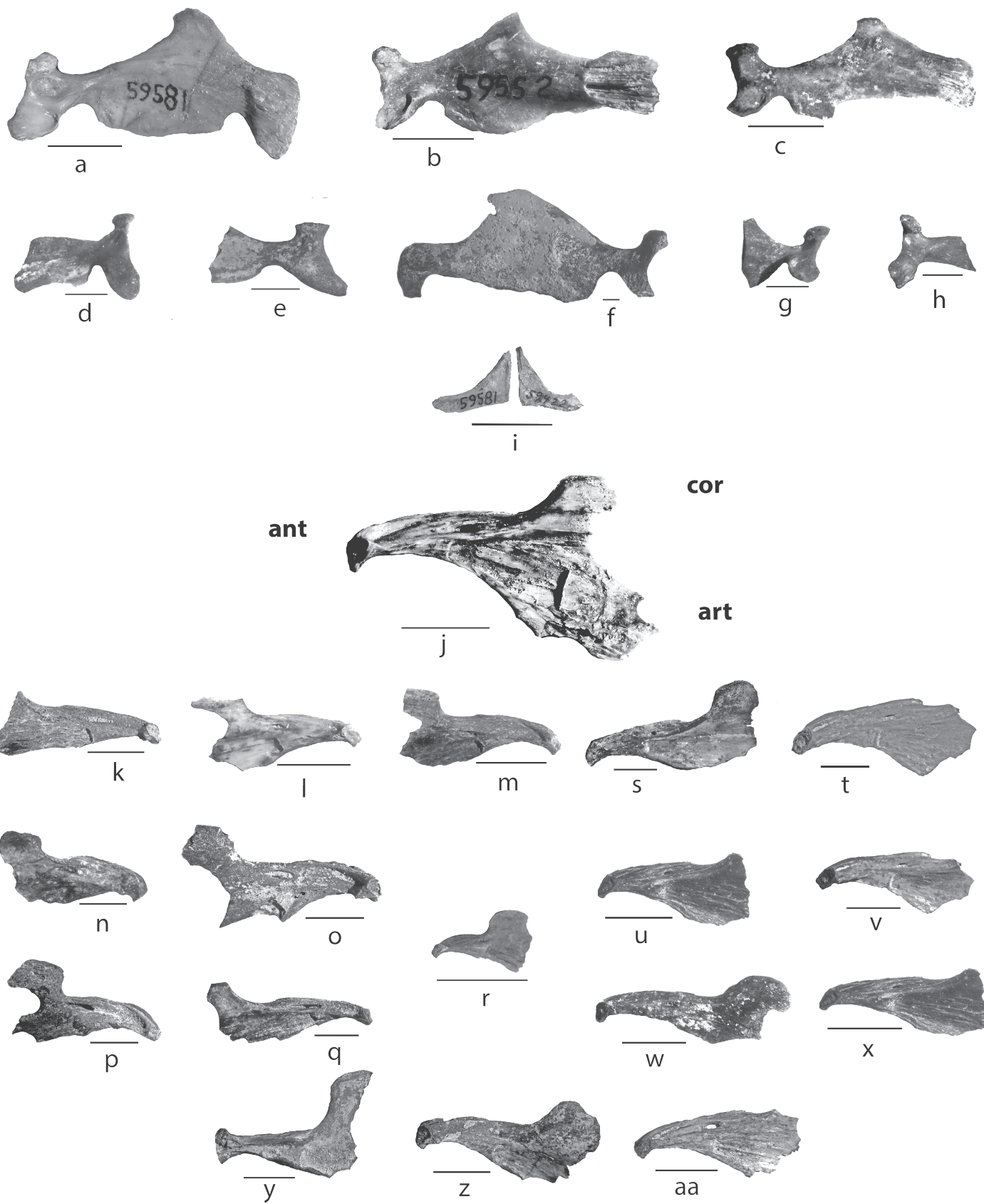
between F_1 hybrids and parents, in addition to being hyper-variable (Hubbs et al., 1943), as expected from Anderson's (1949) studies of hybrid plants. Introgressed samples show multiple individuals with uncorrelated and mixed characters in a gradation between the parental forms.

Intergeneric fish hybrids often had abnormal gonads and presumed lower fertility, lending weight to hybrid interpretations. Identities of hundreds of hybrid suckers in the Hubbs collections have been confirmed by subsequent morphological analyses (Miller and Smith, 1981) and DNA sequence analysis (Dowling et al., 2016). This paper attempts to look at several lines of evidence—morphology, paleontology, multivariate analysis of trait distributions, spawning and feeding ecology, analysis of DNA sequence variation, and an example of gonad histology and hybrid intersexes, to develop a broader perspective on the complex evolution of *Chasmistes, Deltistes,* and *Xyrauchen*.

METHODS

We assembled data on catostomid hybrids from tables in Hubbs et al. (1943), Hubbs and Hubbs (1947), and Hubbs and Miller (1953) who recorded measurements from hundreds of catostomid hybrids collected in nature. These are presented here as biplots of two measurements with the third measurement represented as symbol color, using the method of Anderson (1949) (Fig. 11). Scatter plots included all individuals of two species and intermediates in each wild-caught sample. To develop a more appropriate test of hybridization and introgression, the method of Neff and Smith (1979) applies Principal Component Analysis to meristic and measured variables of Lake Suckers and *Catostomus* Figs. 10, 12a, 12b). Comparison of these analyses to patterns of Principal Component studies of lab-reared hybrid sunfish and minnow progeny validates the method for testing for introgression. These experiments and later statistical studies by Bookstein et al., 1985, established the practical and theoretical validity

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Figure 8.— Variation in mesial views of one local sample of fossil maxillae, **a - h**; premaxillae; **i**, and dentaries; **j – aa**, from Pliocene Glenns Ferry Lake. Scale bars 1 cm. Anterior to the left in a, b, c, h, j, r - aa. Anterior (ant) ends of dentaries and maxillae are robust, strong, and unbroken; the coronoid (cor) process for muscle attachment to the dentary is usually broken; the articular-angular (art) is usually detached from the dentary. The anteroventral (premaxillary) process of the maxilla varies excessively, possibly because of introgression and mouth movements influencing growth. The relative length and curvature of the anterior process is the most variable part of the dentary. Dentary r, 1.2 cm long, is from a juvenile, with typical *Chasmistes* shape nearly fully developed at a body length of about 16 cm. Maxilla g has the perpendicular angle of the premaxillary process expected of a hybrid with *Catostomus*. None of these dentaries resemble *Catostomus* or hybrids.

of Principal Component Analyses for distinguishing hybrid fishes from parental species and for discovering evidence of introgression.

We studied morphological traits of museum wet specimens, skeletons, fossils, and mtDNA from all known species and fossils throughout the range of Lake Suckers, including representatives of sympatric *Catostomus*. Fossil samples include 213 cataloged lots of *Chasmistes* and 60 catalogued lots of *Deltistes* mostly from the Pliocene Glenns Ferry Formation in Owyhee County, Idaho, but also from Washington, Oregon, and California. The catalogued lots in the University of Michigan Museum of Paleontology have one to dozens of bone specimens each from over 30 localities reported and mapped in Miller and Smith (1981).

Gill-nets were used to obtain a mixed sample *Chasmistes*, *Catostomus*, and hybrids from Palisades Reservoir, Bonneville County, Idaho, in 2015 and 2016. Specimens were sorted on the basis of external traits in the field and tissues of subsamples were placed in 95% ethanol and RNAlater to characterize their DNA; whole specimens for later dissection and analysis were preserved in appropriate fluids or frozen (Tables 1—3). Gonads of 29 specimens were dissected, fixed in Davidson's solution, stained with hematoxylin and eosin, embedded, sectioned, and examined for abnormal spermatogenesis or oogenesis by Justin Schilling (Fig. 13).

Molecular Analysis.— Whole genomic DNA was extracted from muscle or fin tissue of 13 individuals thought to represent the range of variation in parents and hybrids from Palisades Reservoir. Fin clips were digested as described in Dowling et al., (2005) and DNA was isolated using a ratio of 1.8:1.0 AxyPrep paramagnetic beads (Axygen, Inc.) to tissue lysate. This was followed by two washes with 70% ethanol while beads were bound to a magnet. Purified DNA was eluted in ultrapure water.

We characterized sequence variation using two mitochondrial genes, cytochrome *b* (cyt*b* – primers LA and HA: Schmidt and Gold, 1993) and subunit 2 of NADH dehydrogenase (ND2 – B2$_{Gila}$ and E$_{Gila}$: Schwemm, 2006). Fragments were amplified through 25 cycles of 94 C for 1 min; 48 C for 1 min; and 72 C for 2 min. Samples were sequenced and aligned as described in Dowling et al., 2016 (Genbank numbers: KJ441278, KJ441283, KJ441329, KJ441334, KU697914-KU697916, KU697922-KU697925, KU697927, KU697928, KU697930-KU697934, KU697942-KU697944, KU697950-KU697953, KU697955, KU697956, KU697958-KU697922, MH603006-MH603045). Samples of 14 additional catostomid taxa from other lakes and rivers were studied to provide context and genetic distances for calibration with nine *Catostomus* fossil ages (Table 5).

Phylogenetic analyses of mtDNA sequences were completed using MEGA 7.0 (Kumar et al., 2016). Neighbor-joining trees were constructed from p-distances and strength of nodes examined using 1000 bootstrap replicates. Maximum likelihood analysis used a discrete Gamma distribution

Table 1.— College of Idaho Museum Catalog numbers with lip, jaw, and gill raker measures of Palisades suckers.

CIDA	TL	gap wi	l lip ln	plicae	l jaw ln	u jaw ln	snout ln	head ln	angle	branches
113,388	271	2.1	5.5	0	21.3	12.7	21.7	48.6	19	11
113,390	479	0	11.1	2	31.2	19.7	40.5	92.5	35	15
113,391	373	0	9.1	2	24.2	20.9	35.2	73	22	11
113,392	431	1	11.1	1	28	19.3	41.4	86.8	36	21
113,394	328	1.6	9.1	2	18.7	15.3	27.4	69.6	28	12
113,396	421	3.1	11.5	0	25.5	15.7	40.2	81.1	33	15
113,398	399	1.3	11.1	2	23.4	20.4	33.5	75.2	26	15
113,400	463	2.2	15.7	2	34.1	20.8	44.3	99.3	37	30
113,401	374	1.8	8.6	2	22.2	18.1	32.2	68	26	7
113,402	371	1.3	9.6	1	10.2	19.4	31	71.9	29	19
113,403	347	1.9	9.1	2	19.6	14.1	31.2	65	23	11
113,405	385	2.5	6.5	2	24	18.2	33	72.4	31	22
113,406	407	0	10.7	2	24	23.2	36.2	80	33	23
113,408	480	2	11.1	0	34.3	21.6	45.3	95.3	37	28
113,410	420	1.7	12.1	2	31	24.4	39	87.7	27	28

to model evolutionary rate differences among sites (five categories, [+G], parameter = 1.4392, estimated from the data). The rate variation model allowed for some sites to be evolutionarily invariant ([+I], 56.5552% sites). All positions containing gaps and missing data were eliminated and relative strength of nodes assessed through bootstrap analysis (1000 replicates) (Fig. 2).

Morphological and Osteological Analyses.— Osteological study was facilitated by Ridewood preparations (Bemis et al., 2004) at the University of Michigan. This involved removal of

the right side upper and lower jaw bones, quadrate, symplectic, pterygoid series, hyomandibula, and opercular series from the cranium for clearing with potassium hydroxide and glycerin and staining with alizarin red dye. These bones have more discriminating power than external shape traits (Smith, 1975; Miller and Smith, 1981; Smith et al., 1982; Smith, 1992a), being independent of convergent selection for external hydrodynamic efficiency. Ridewood preparations permit separate examination of nearly whole specimens. Osteological traits were statistically compared with fossil and recent

Table 2.— Measurements of 11 mouth and jaw traits on 17 suckers from Palisades Reservoir and four comparative specimens. Column 1, field identification and number: LS, Lake Sucker; US, Utah Sucker; **X**, hybrid; Column 2. **gap**: 0, none; 1, intermediate; 2, wide; **den vert ln**: dentary vertical length, mm; **den dist ln**: dentary distal length, mm; **den long ln**: dentary longitudinal length, mm; **den angle**: dentary angle degrees; **max ln ext**: maxillae length exterior, mm; **max ln int**: maxillae length interior, mm; **max angle**: maxillae angle degrees; **pmx height**: premaxillae height, mm; **pmx width**: premaxillae width, mm; **pmx angle**: premaxillae angle degrees. See Fig. 5, image of jaw bones from individual fish.

	gap 0,1,2	den vert ln	den dist ln	den long ln	den <	max ln ext	max ln int	max<	pmx height	pmx width	pmx<	in Fig. 5:
1 LS470	2	24	12	23.2	118	26.6	23.4	117	—	—	—	
2 LS464	2	24.6	14.5	24.7	125	28.6	25.2	100	—	—	—	
3 LS480	2	23	8.9	20.2	119	27.8	21.5	114	14.2	9.2	93	Fig. 5a
4 LS 919	2	7.5	3.3	6.9	130	7.7	4.3	113	7.5	6	93	Fig. 5b
5 LS 920	2	11	6	9.4	110	11.4	10.2	98	8	4.5	94	
6 LS 952	2	14.5	6.4	14.2	114	17.3	14	113	9	5	93	Fig. 5d
7 US 122	0	13.9	8.3	14.5	125	16.5	15	114	9	6.9	106	
8 US 1225	0	17	8.5	15	116	19.5	17.5	113	12	7.1	90	Fig 5c
9 US 1237	0	10	5.5	9.6	103	12.2	11	116	5.2	5	110	
10 US 1243	0	10	4.6	10	114	11.6	10	100	7	5	98	Fig. 5g
11 US 145	0	10	4.9	10	105	12.5	10.5	97	6.5	4.6	106	
12 US 249	0	20.7	10.8	8.2	116	22.4	20	124	13.3	9.5	93	Fig. 5i
13 X 1140	1	17.3	9	14.5	117	18.5	15.9	110	9.1	6.5	93	Fig. 5h
14 X 1020	1	10.1	4.1	9.3	118	11.2	10.1	100	8	4.5	—	
15 X 933	1	12.6	5.1	11.2	110	14.2	12.9	105	5	4	98	
16 X 943	1	14	6	12.3	103	14.9	13.2	110	5	4	96	
17 LS401	2	25	13	12.2	130	25.5	22.2	114	—	—	—	
1type muriei	2	24	12.1	22.5	124	28.5	21.5	117	14	10	—	UMMZ81530
old liorus	2	26.3	12.5	24	121	26.1	24.4	100	13.2	11.5	103	USNM20389
spatulifer	2	30	14.1	40	126	36	36.4	135	12.5	19	105	UMMP59581
ardens	0	23.5	12.3	21.5	120	25.2	22.4	93	12.2	8.4	90	UMMZ183687

Chasmistes, Deltistes, Xyrauchen, and *Catostomus* in the Museum of Paleontology and Museum of Zoology, University of Michigan (Fig. 7). Correlations (Pearson) among bone measurements were calculated to test genetic and ontogenetic independence (Table 4).

Ridewood dissection destroyed some of the right half of the lips of the Michigan specimens; therefore the undissected subsample of specimens at the College of Idaho was photographed and their lips, gill-raker numbers, gill-raker branching, and external traits evaluated. Specimens were measured with dial calipers and photographed with a Dino-Lite microscope and camera or iPad camera. Multivariate statistical analyses, particularly Principal Component Analysis, were used to ordinate correlated associations of individual fish values: A Pearson correlation matrix was calculated from the variable-by-variable matrix. Principal components based on Eigenvectors of the variable-by-variable correlation matrix give us new, orthogonal variables, a few of which carry most

of the correlated information in the original variables. The original variable-by-specimen data matrix, when multiplied by the Eigenvectors, produces an ordination of each case on the principal components, those with the highest variance explained permit scatter plots that display similarities of individual fishes on biplots of the main axes. Principal Component Analysis (PCA) is the preferred statistical method for analyzing data when hybrids are suspected (Bookstein et al., 1985) because all the data, without prior classification, contribute to discovering whether or not the population is bimodal. The scatterplot of the ordination shows whether the variability of the intermediates indicates backcrossing, a separate species, or one species with ecophenotypic variants (Fig. 11; Neff and Smith, 1979). PCA in the present paper was performed with XLStat software (Addinsoft, 2016).

Measured specimens and traits in the several subsamples include a geographically broader sample of fossil and modern neurocrania (Fig. 9 from University of Michigan Museum
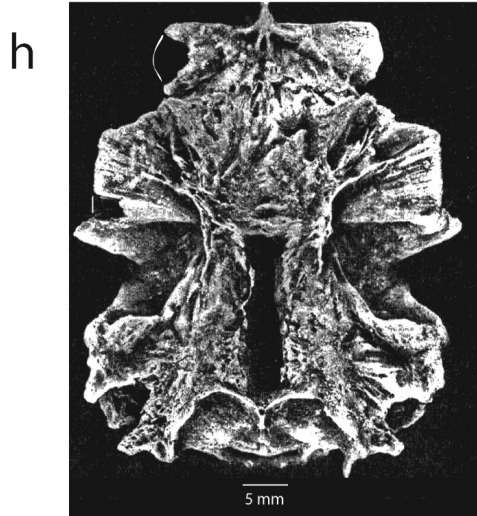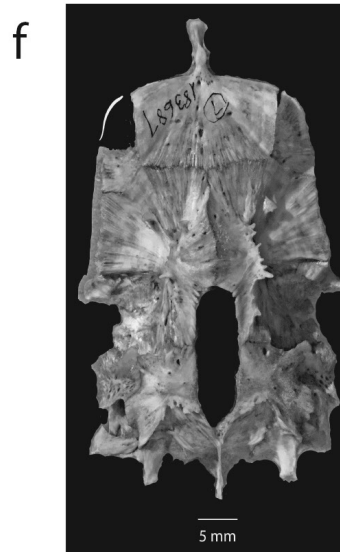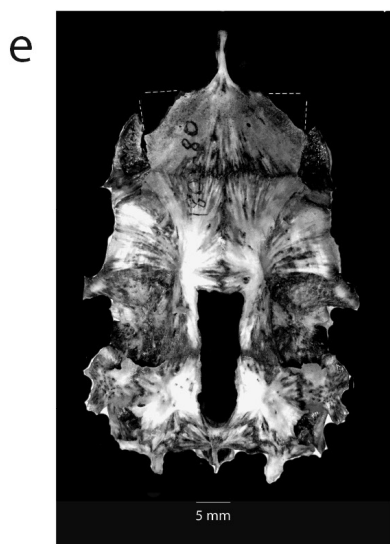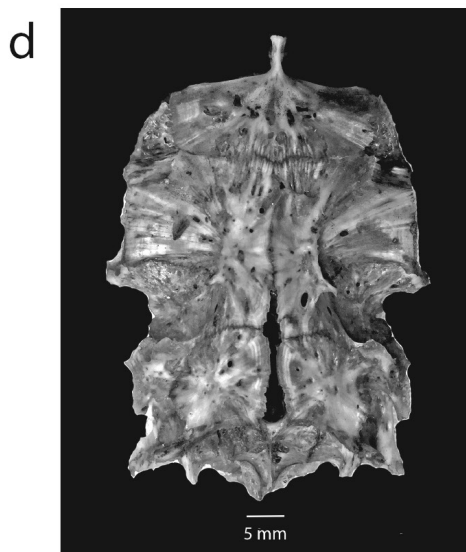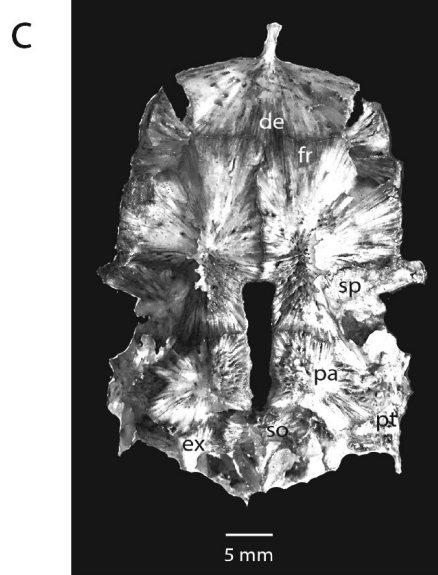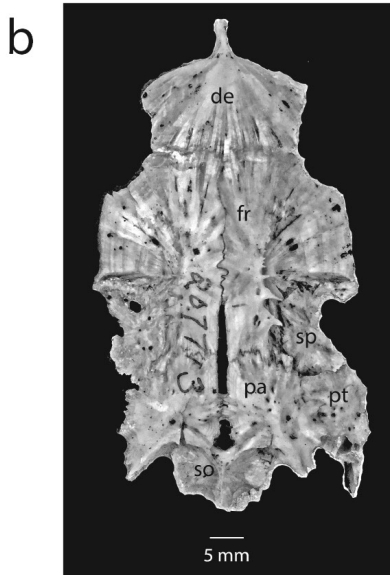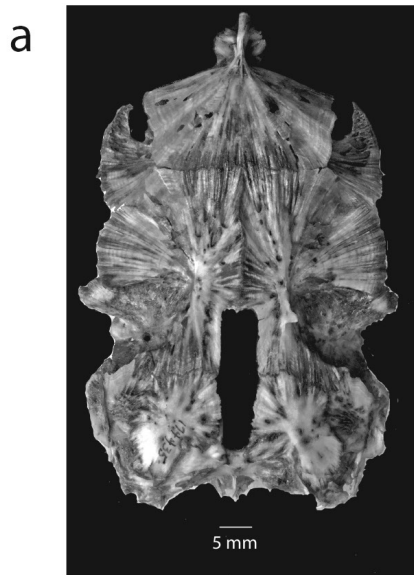
of Zoology and Museum of Paleontology), given in Tables 1, 2, and 3, and shown in the associated scatter-plots. These analyses permit placement of Palisades Reservoir, other Lake Suckers, and fossils according to their shapes in the comparative context of other *Chasmistes* and their sympatric *Catostomus*. The prediction is that morphotypes of *Catostomus* vs. introgressive hybridization progeny between *Chasmistes* and sympatric *Catostomus* will be apparent from the number of clusters—bimodal, graded, or mixed unimodal distributions of traits and individual specimens in Principal Component ordinations (Figs. 10, 12a, and 12b, as in Fig. 11).

Historical, fossil, and other specimens from University of Michigan Museum of Paleontology and Museum of Zoology were also included in analyses to evaluate changes through geological time. Early 19th and 20th Century *Chasmistes* borrowed from other museums are included to test whether traits indicate more introgression relative to modern specimens. These include *Chasmistes brevirostris*, ANSP 20959; and *Chasmistes liorus liorus*, USNM 27361 (Miller and Smith, 1981, figs. 6A and 7A). The fossils and 19th Century samples, from different fluvial vs limnetic habitats, help establish whether there is a temporal trend toward more frequent hybridization among divergent phenotypes representing the same species. Palisades Reservoir suckers provide comparative material from 2015 and 2016 samples taken from the small, recently invaded reservoir.

Age Calibration.— The phylogeny of *Catostomus* and Lake Sucker cytb and ND2 mtDNA (Fig. 2) shows two clades, *Catostomus catostomus* and its sister group including all other *Catostomus*, which we informally refer to as the 'large-scale clade.' The oldest fossil *Catostomus* positively identified to be in the large-scale clade is from sediments of Pickett Creek,
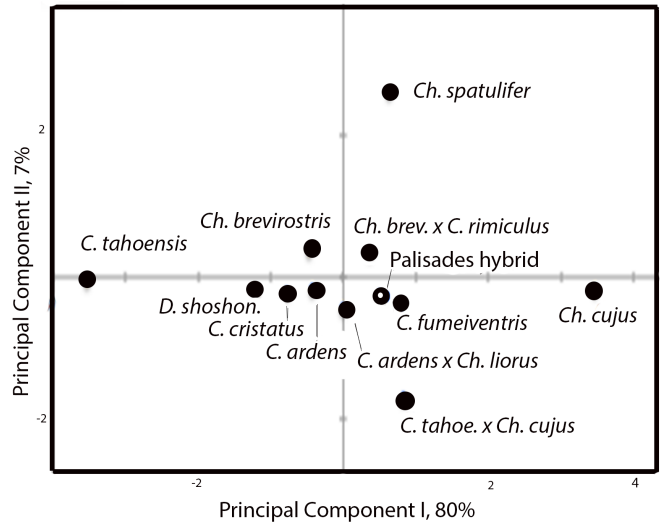

Figure 10.— Ordination of 12 neurocranial measurements of 12 taxa in Table 3 and Figure 6 on Principal Components I and II. Variables are given in Table 3 and Figure 5. Size measures load heavily on Component I; dermethmoid shape and width measures contribute to Component II. All specimens near the center of the plot are introgressed mixtures between *Chasmistes* and *Catostomus*. The unimodal scatter is consistent with the introgression hypothesis.

Owyhee County, Idaho (age 10.5 – 8.5 Ma, Carpenter and Smith, this volume; Buechler et al., 2007; Buechler and Smith this volume), with fossil age inferred to be 9.5 Ma based on the bounding radiogenic ages of two volcanic ashes at Pickett Creek, which have compositions associated with the Twin Falls volcanic field, 10.5 - 8.5 Ma. Slightly older *Catostomus* fossils from the Oregon-Idaho Graben are not unambiguously identifiable as *Catostomus*. The calibration procedure uses the density of the *Catostomus* fossil record among 1 m.y. bins (horizons) to calculate an extended age correction factor (Marshall, 1990). We use Marshal's alpha for $C_{.50}$ to correct the fossil age for each estimate of the most recent gene exchange between two populations, given the assumption that our oldest fossil is unlikely to represent lineages immediately following the split (See Results) or introgression (Marshall, 1990).

RESULTS

Mouth and Lips.—Adult *Chasmistes* mouths were originally subterminal, with plicae (ridges, Fig. 3c) not papillae, on the lips. The mouth of *Chasmistes* was originally terminal, moved anterodorsally by elongation of the lower jaw (Fig. 3d; Fig. 7; Fig. 8) and apparently by a lifetime of feeding while swimming with the mouth open, directing the gape toward plankton. This was one of the most distinctive traits of *Chasmistes* compared to other catostomids, which usually have the mouth directed ventrally for feeding on benthos; *Chasmistes* mouths now are variably subterminal. The subterminal mouth

Figure 9.— *Catostomus*, *Chasmistes*, and hybrid neurocrania. **a**, *Chasmistes cujus*, Pyramid Lake, NV, UMMZ 174435; **b**, *Chasmistes cujus × Catostomus tahoensis*, Pyramid Lake, NV UMMZ 207713; **c**, *Chasmistes × Catostomus fumeiventris*, Owens Valley, CA, UMMZ 181667; **d**, *Chasmistes brevirostris*, Copco Reservoir, CA, UMMZ 180616; **e**, *Chasmistes liorus × Catostomus ardens*, Utah Lake, UT, UMMZ 180680; **f**, *Catostomus ardens*, Cottonwood Creek, UT, UMMZ 183687; **g**, *Catostomus cristatus*, Glenns Ferry Formation, ID, UMMP 62400; **h**, *Chasmistes spatulifer*, Glenns Ferry Formation, Idaho, UMMP 58210; **i**, *Deltistes shoshonensis*, Glenns Ferry Formation, ID, UMMP 59605. Bone identifications (on panels b and c), **de**, dermethmoid; **fr**, frontal; **sp**, sphenotic; **pa**, parietal; **pt**, pterotic; **so**, supraoccipital. a, b, c, illustrate parental forms and hybrids in the Lahontan Basin and Owens Basin. *Chasmistes fumeiventris* has the wide dermethmoid bone of *Chasmistes cujus*, commensurate with their shared DNA; d, *Chasmistes brevirostris* has DNA and mixed morphological traits of *Catostomus rimiculus* of the Klamath Basin; e, hybrid *Chasmistes* from Utah Lake, shares DNA and some morphological traits of *Catostomus ardens*, f. a wide dermethmoid is diagnostic for *Chasmistes*.

Table 3.— Twelve neurocranium measurements on 12 catostomin specimens including hybrids. **de**: dermethmoid. **ln**: length (mm). **wi**: width (mm). **pf**: prefrontals. **fr**: frontal. **sp**: sphenotic. **pa**: parietals. **nc**: neurocranium. **diag**: diagonal length (mm). **pt**: pterotic. **poros**: porosity rating: 0, low; 1, intermediate; 2, high.

Specimen	de ln	de wi	pf wi	fr ln	fr wi	sp wi	pa wi	nc ln	pa ln	pa diag	pt wi	poros
Ch. spatulifer* UMMP 58210	8	27.5	40	29.9	22.2	24.8	31.3	59.2	7	19.7	43	2
Ch. cujus UMMZ 174435	20.3	35.5	56.7	36.3	29	30.2	39.2	87.2	17.3	25.1	60.2	2
Ch. brevirostris UMMZ 180616	11.1	25	32.1	20.9	17.6	19.6	24.2	53	10.2	16.3	34	1
C. fumeiventris UMMZ 181667	14.1	25.3	35.3	29.7	19	25.1	29.2	62.4	11.8	18.9	41.5	1
Palisades hybrid sucker UMMZ 250302	13	21	31	22.2	19	25.1	38	58.6	11	21	46	1
C. ardens UMMZ 183617	12.5	20.6	22.8	23.5	17.5	21.5	26.2	56.2	10.3	15.8	36.1	1
C. tahoensis UMMZ 169022	6.5	10.1	17.5	13	8.5	10.1	12.8	27.3	3.8	7.2	19.5	0
C. cristatus* UMMP 62410	14.4	17	29	23.3	16.1	18.8	21.6	56.7	6.5	15.3	34.2	1
Deltistes shoshonensis* UMMP 59605	12.2	13.7	15.2	24.9	15.9	16.4	19.1	56.3	8.1	14.4	33	1
Ch. liorus mictus UMMZ 180680	14.5	21.5	35	24.2	17.2	22.9	24	62	10.7	16.2	39	1
Ch. cujus x *C. tahoensis* UMMZ 207713	15	23	40	28.8	21.6	26	33.2	68	11.6	19.8	46	0
Ch. brevi. x *C. rimicul*. UMMZ 180686	12	28.4	44	18.9	22	24.7	25	60	11.8	18.2	39	1

*fossils

is acquired after the first year of life; larval catostomids have ventral mouths, but juvenile *Chasmistes* as small as 32 mm have subterminal mouths (Miller and Smith, 1981, fig. 1). The uplifted profile of the snout of adult *Chasmistes* is no longer a reliable character (Miller and Smith, 1981, figs. 4A, 6A, 7A) because introgression has shortened the jaw bones and many of the fishes have fed on benthos, where papillae are useful. Snouts are now rounded or only slightly elevated, more like *Catostomus* or hybrids (Miller and Smith, 1981, figs. 6B, 7B, and 9A, B, C) but never like relatively purer *Chasmistes* from over 100 years ago, which had longer jaw bones, with mouth openings elevated to terminal position, about one eye diameter below the level of the eye (Miller and Smith 1981, figs. 4A, 6A). Subterminal-mouthed *Chasmistes* no longer exist in nature; specimens most similar to original form are in Pyramid Lake, Nevada.

Catostomus, Xyrauchen, and *Deltistes* have ventral mouths and papillose lips. Papillae (Fig. 3a) are small, rounded elevations bearing taste buds. Lips of *Chasmistes* are now (Twenty-first Century) more variable than they were in the Nineteenth Century. Mouths of *Ch. muriei* in the Upper Snake River drainage range from ventral and papillose, like *Catostomus* (Figs. 3a, b; Figs. 4g, h) to sub-terminal and plicate as in hybrids between *Chasmistes* and *Catostomus* (Figs. 3c, d; Figs. 4b, d; Miller and Smith, 1981, figs. 7B, 7C). The gaps between the lower lip lobes are variable in *Chasmistes* of Utah Lake, Upper Klamath Lake, and the Snake River drainage. Over 400 suckers caught in Palisades Reservoir in 2016 had gaps of variously intermediate widths between the bases of lobes of the lower lips (Fig. 3c; Fig. 4d; Miller and Smith 1981,

figs. 2 and 4D). The lower lip gap is an external diagnostic trait that discriminates Lake Suckers from *Catostomus* species, except where introgression has occurred. Papillae on the ventral surfaces of the lower lips (Fig. 3a; Figs. 4g, h) are not prominent in *Chasmistes* or hybrids, but are flattened, smoothed over, elongate, and longitudinally oriented, grading into plicae (ridges) (Fig. 3c.; Figs. 4a-f). This lack of papillae in Nineteenth Century *Chasmistes* is in contrast with the small, mound-shaped papillae typical of *Deltistes, Xyrauchen*, and all *Catostomus* in North America (Fig. 3a; Figs. 4g, h), but is now mostly gone, except in Pyramid Lake.

Gill Rakers.— Gill rakers form a sieve in the branchial basket of suckers; the sieve is fine-meshed in *Chasmistes* (Fig. 5c), intermediate in *Xyrauchen* (Fig. 5b), and coarser-meshed in *Catostomus* (Fig. 5a; Willink, 2002). *Chasmistes* species originally had 40 - 53 gill rakers on the first arch. *Xyrauchen texanus* has 36 - 50 gill rakers, depending on body size (Hubbs and Miller, 1953). *Deltistes luxatus* has 23-37 gill rakers (Markle, 2016). *Catostomus ardens* has 28 - 34 gill rakers on the first arch; hybrids between *C. ardens* and *Ch. liorus* from Utah Lake are intermediate and highly variable, 30 - 47 (Miller and Smith (1981, table 3). Gill rakers of Palisades Reservoir specimens are intermediate between *C. ardens* and *Ch. muriei*, but within the range of the hybrids between *Catostomus ardens* and *Ch. liorus* in Utah Lake. The type specimen of *Ch. muriei* from Jackson Lake has 40 gill rakers on the first arch. Here we focus on the numbers of branches—a more diagnostic trait than numbers of gill rakers.

Chasmistes gill rakers are diagnosed by numerous distinctive branches (Figs. 6a, b, f) called tufts by Gilbert
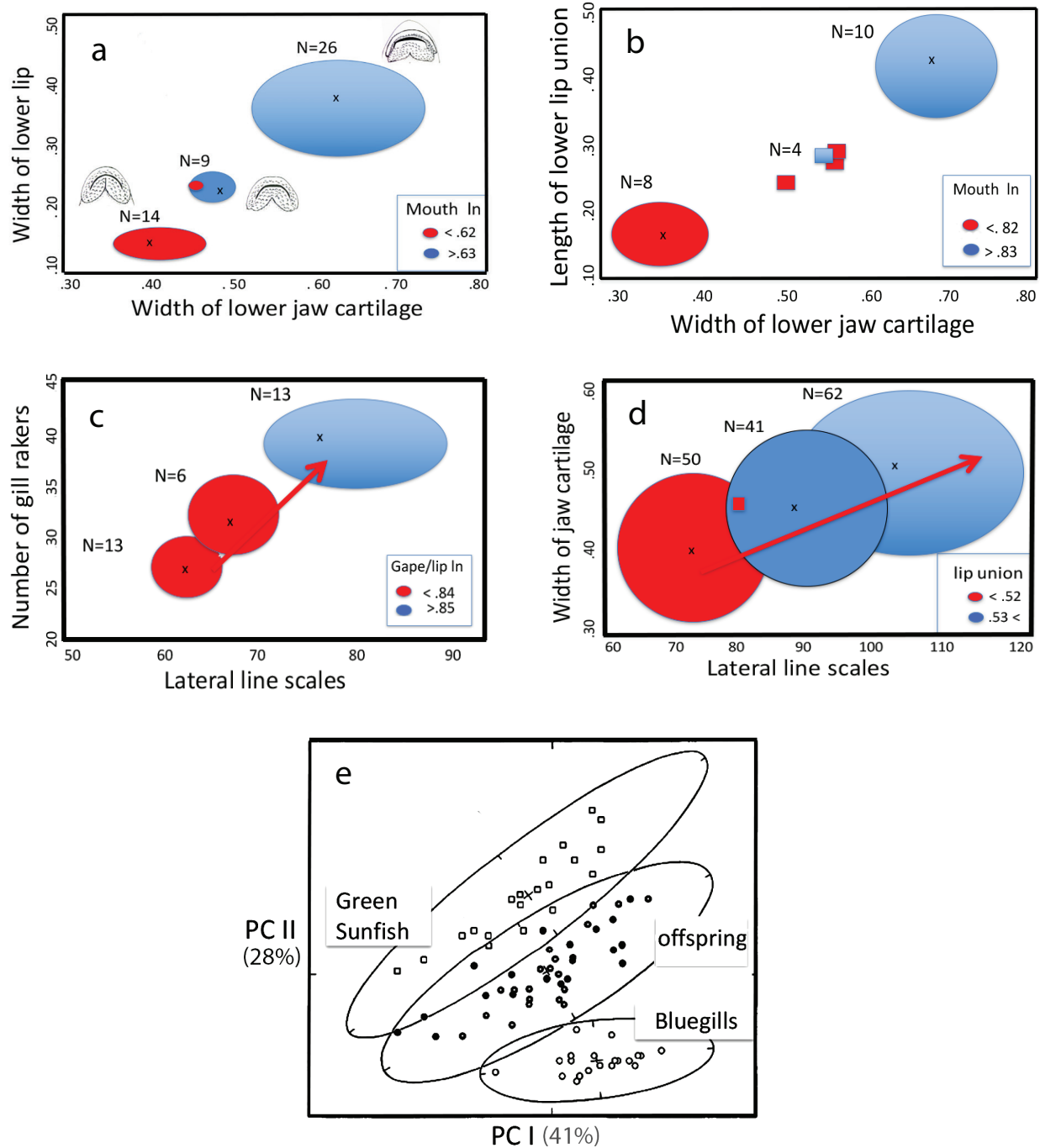
Figure 11.— Scatter plots of data from Hubbs et al., 1943, and Hubbs and Miller, 1953. Boundaries of ellipses from tables of population statistics transposed to thousandths of Standard Length. **a**, *Catostomus tahoensis* (N=14), *Pantosteus lahontan* (N=26) and nine hybrids. Colors represent mouth length below 0.62 (red) or above 0.63 (blue). Limited variation and intermediacy in the nine hybrids implies that they are F1s. **b**, *Catostomus insignis* (red), *P. clarki* (blue), and hybrids. Limited variation and intermediacy in the four hybrids implies that they are F1s. **c**, *Catostomus macrocheilus* (red), *P. columbianus* (blue), and hybrids. Hypervariable and non-intermediate hybrids suggest a history of introgression. Later analysis of mtDNA by Dowling, et al., (2005) and Unmack et al. (2013) discovered *C. macrocheilus* mtDNA in all *P. columbianus*, demonstrating past introgression. **d**, *Catostomus insignis* (red), *Xyrauchen texanus* (blue), and hybrids. Measurements from Hubbs and Miller, 1953. Hypervariable and non-intermediate hybrids suggest a history of introgression. Analysis of mtDNA (Dowling, et al., 2005; Unmack et al., 2013) discovered *C. insignis* mtDNA in *Xyrauchen*, demonstrating past introgression. **e**, Principal Component Analysis of measurements and meristic counts of experimentally produced hybrids of Bluegill and Green Sunfish demonstrate general but variable intermediacy of a large sample of hybrid progeny (from Neff and Smith, 1979). Variability of parents and hybrids suggests past introgression, consistent with normal field observations of Green Sunfish.
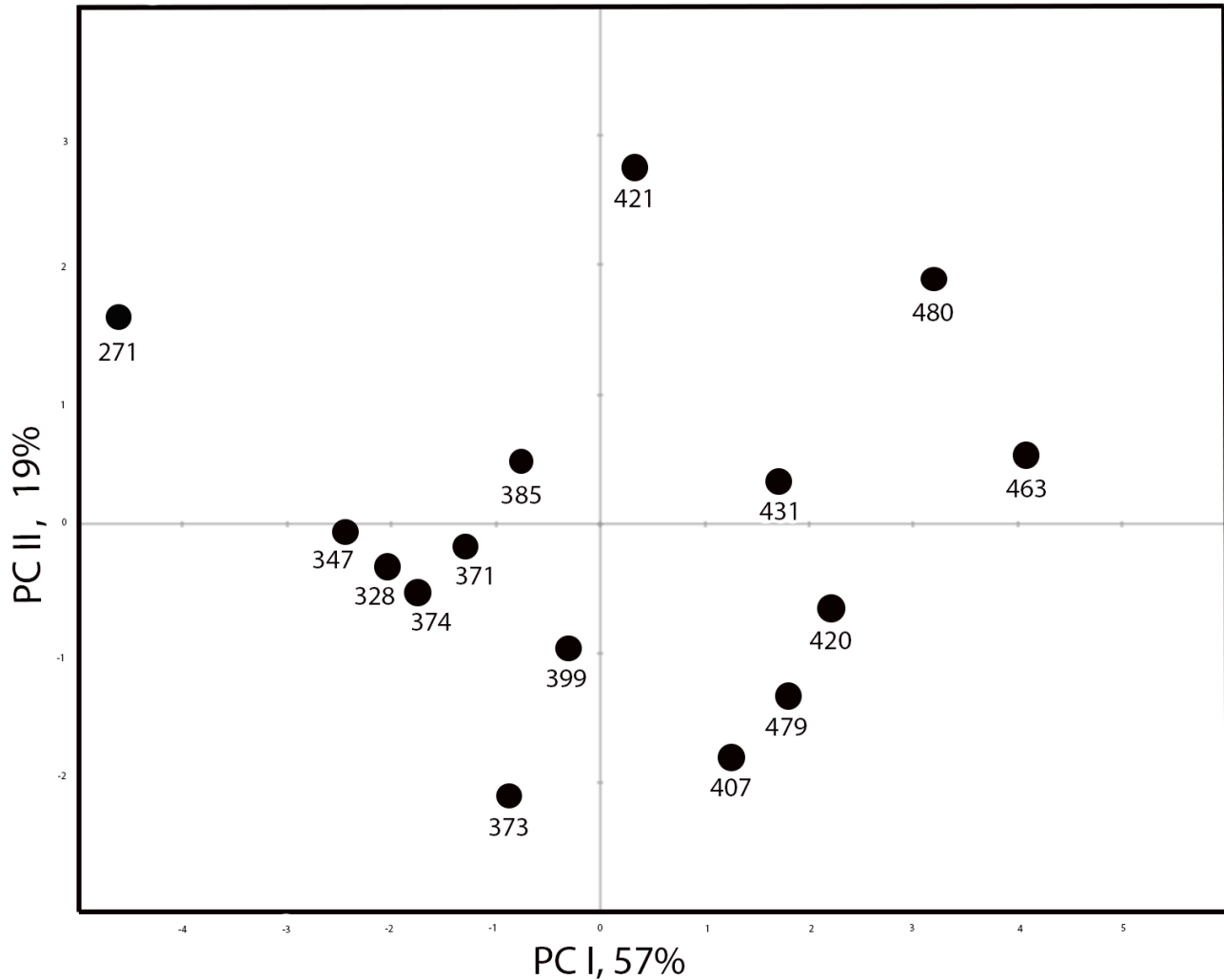
Figure 12a.— Principal Component Analysis of nine external traits of Palisades Reservoir Suckers. Position on principal component I correlated with body length. On the left, 271 is *Catostomus ardens*; all others are Palisades suckers. Ordination of black filled round symbols is based on scores of 15 specimens on measures known to differentiate the two parental phenotypes (Table 2). Specimens do not cluster into two or three phenotypes on axis I × II, as expected if specimens were either *C. ardens* or *Ch. muriei*; and do not show a progression of phenotypes from *C. ardens* to *Chasmistes*-type on axes I × II, as predicted by the ecophenotype hypothesis. Positions of specimens in common in Figs. 7a and 7b are not correlated, meaning that the characters of the two phenotypes are randomly mixed.

(1889), but *Catostomus* gill rakers have no branches (Fig. 6d); hybrids, including most fishes examined from Palisades Reservoir, have branches, but fewer than half as many on each gill raker. The high number of gill-raker branches emanating from a bony plate on each raker of *Chasmistes* is the most apomorphic trait of the genus (Figs. 6e, g, h)—the bony plate and its branches are shared in *Moxostoma* and *Chasmistes* among Catostomidae (Figs. 6a - h; Miller and Smith, 1981, figs. 5A, 8A', C') but the profuse branching in non-introgressed *Chasmistes* is unique (Fig. 5c; Figs. 6a, b; Willink, 2002). The bony plates (Figs. 6a, e-h), are distinctive enough to have been found as fossils (UMMP 118180) in MioPliocene Mono Lake sediments by Ken Gobelet (Fig. 6h). The Palisades suckers

have fewer branches, their numbers ranged from 11-30 per gill raker in Palisades suckers (Figs. 5a - c), as opposed to about 60 per raker in relatively non-introgressed *Chasmistes* (Miller and Smith, 1981, figs. 5A, B', C'). Gill-raker branch numbers of hybrids, including Palisades specimens, are variable and intermediate between *Chasmistes* and *Catostomus* (Miller and Smith, 1981, figs. 5D, 8B, B', C, C').

Jaw Osteology.— Osteological traits of 19th Century and Pliocene Snake River fossil *Chasmistes* (Fig. 8) are distinct from others (Miller and Smith, 1981, figs. 10A, D, G; 11 A, B, C, D). The bones of the upper and lower jaw of *Chasmistes* from Palisades Reservoir (Figs. 7a-e and 7g-i) are variable and intermediate between bones of the type specimen of
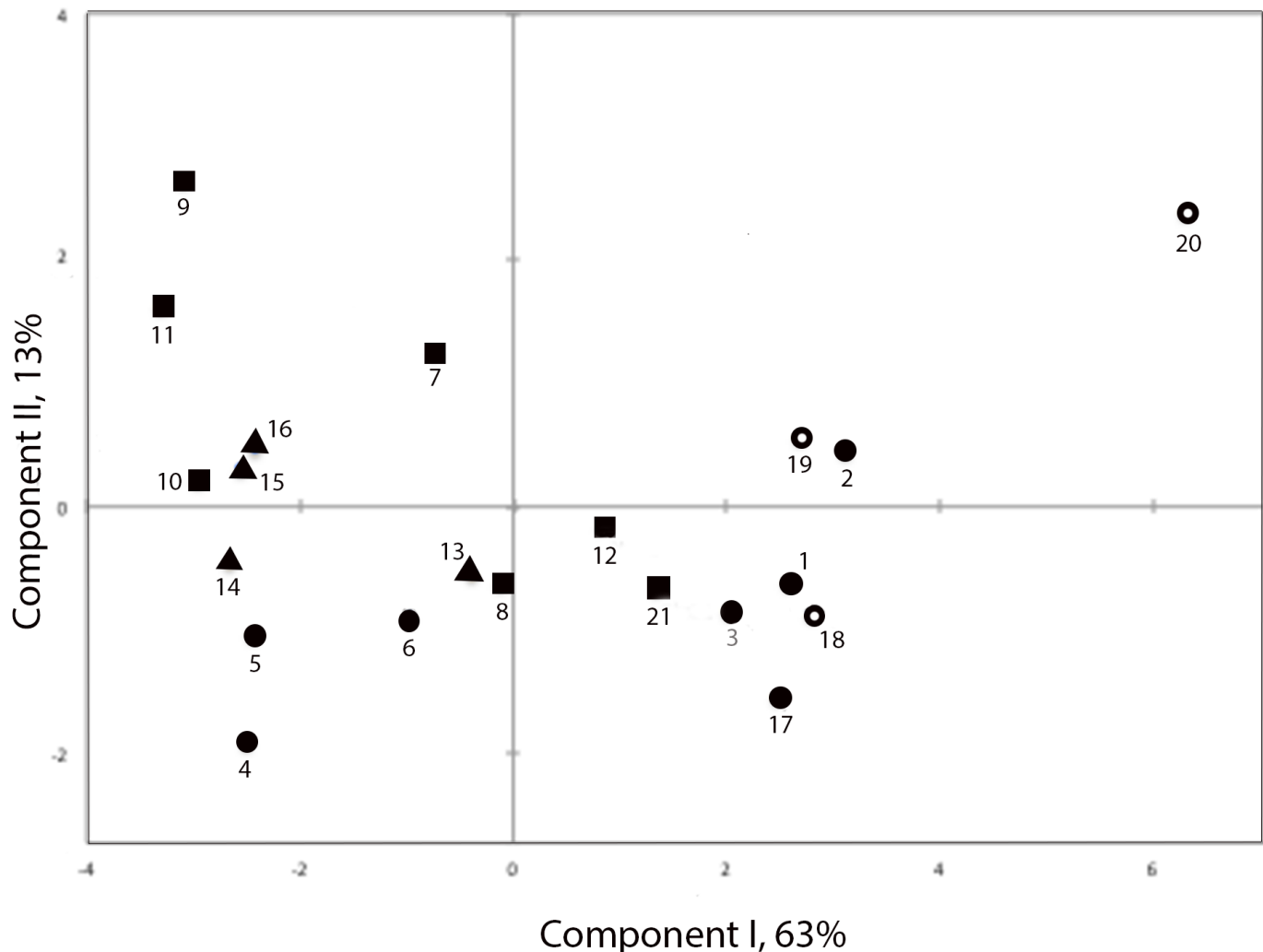
Figure 12b.— Principal Component Analysis of 11 bone and lip measurements on 21 suckers sampled from Palisades reservoir. Provisional field identifications: round symbols, Utah Suckers; squares, *Chasmistes*; triangles, hybrids; 18, type specimen of *Ch. muriei*; 19, early specimen of *Ch. liorus*; 20, fossil *Chasmistes* from Glenns Ferry Lake; 21, Utah Sucker from the Bonneville Basin. The data suggest that many or most specimens of Utah Suckers and Lake Suckers in Palisades Reservoir contain mixtures of genes, as did the type specimen of *Ch. muriei*. Component I is correlated with size. Component II shows divergence of *Chasmistes* phenotypes with growth, and *Catostomus* becoming more like Lake Suckers with growth, suggestive of the ecophenotype hypothesis, but a transformation is not evident. The unimodal scatter of specimens justifies rejection of the hypothesis that there are genetically pure individuals of two parental species in this analysis.

Ch. muriei and of *C. ardens* (Miller and Smith, 1981, figs. 11A, 10C), resembling bone shapes in populations that have been introgressed (Table 3) (Miller and Smith, 1981, figs. 10A, B, E, H). Specifically, the antero-median process of the maxilla is not directed antero-mesially (Fig. 8g) as in more typical *Chasmistes* (Figs. 8a - f, h), but more mesially, as in intermediates and *Catostomus* (Figs. 7a - h).

The anterior (distal jaw) part of the dentary of *Catostomus* is shorter, with a more acute angle between the distal and proximal limbs (Fig. 7c), unlike *Chasmistes*, including *Ch. muriei* (Figs. 7a, e; 8j-aa; Miller and Smith 1981, fig. 11A). The Palisades sucker morphologies resemble thoroughly-mixed jaw phenotypes, probably acquired in the 60 years

during which the fish could have been in the reservoir; they seem to be more variable than *Ch. muriei* in Jackson Lake (Fig. 3). The variable-by-variable correlation matrix shows high values for measurements within functional complexes, but not among different tissues or unconnected parts (Table 4). Many of the *Chasmistes* jawbones from the fossil record in California and Oregon show indications of plesiomorphy or introgression (Miller and Smith, 1981).

Neurocrania.— Neurocrania of *Catostomus* and *Chasmistes*, along with other catostomid genera, were compared using geometric morphometrics and two-dimensional orthogonal grids to determine the amounts and complexity of evolutionary shape change. *Catostomus* and *Chasmistes* show large

Table 4.— Correlations among measurements. See Table 2 for variable definitions. Correlation coefficients above .80 indicated in bold font.

Variables	Var1	Var2	Var3	Var4	Var5	Var6	Var7	Var8	Var9	Var10	
v1 gap width	**1**										-0.200
v2 dent v ln	0.42	**1**									-0.126
V3 dent dist ln	0.33	**0.96**	**1**								-0.064
V4 dent dors ln	0.42	**0.83**	0.77	**1**							0.149
V5 dent angle	0.45	0.50	0.55	0.42	**1**						-0.201
V6 max ext ln	0.40	**0.98**	**0.94**	**0.87**	0.50	**1**					-0.072
V7 max mes ln	0.34	**0.96**	**0.93**	**0.90**	0.45	**0.98**	**1**				0.036
V8 max angle	0.19	0.36	0.29	0.35	0.26	0.42	0.43	**1**			0.016
V9 pmx ht	0.40	**0.87**	**0.86**	0.60	0.66	**0.85**	0.78	0.30	**1**		-0.339
V10 pmx w	0.39	**0.87**	**0.84**	**0.88**	0.61	**0.89**	**0.92**	0.52	0.74	**1**	0.134
V11 pmx angle	-0.20	-0.13	-0.06	0.14	-0.20	-0.07	0.04	0.02	-0.34	0.13	**1**

amounts of shape change between them, but modest complexity of the change in parts between them. That is, separate bones, for example, the dermethmoid, differ in the directions of their shape change, but modifications of larger regions of the skull roof dominate (Bookstein et al., 1985, figs. 4.7.8; 4.7.10; Figs. 9, 10; Table 3).

Comparison of skull surface porosity of *Chasmistes* (containing fat for buoyancy in life, Figs. 9d, h) and relative widths of the anterior and posterior bones of *Chasmistes* and others in Figs. 10a, b vs. c, and d vs. f) illustrate substantial evolved differences among genera 4 - 3 million years ago (Smith, 1975, Bookstein et al., 1985). Fossils (Figs. 10g and h), anchor the long-term trend by being more disparate than modern forms. Figures 10a, b, and c illustrate the Lahontan basin problem, where *Chasmistes cujus* shares mtDNA not with sympatric *C. tahoensis*, but with *Catostomus fumeiventris* of the now-isolated Owens Basin, a Pleistocene connective of the Lahontan Basin (Reheis et al., 2002). *Catostomus fumeiventris* bones and gill rakers have *Chasmistes* synapomorphies; *Chasmistes cujus* is most similar to *C. fumeiventris* in external head shape. Figure 9d illustrates the originally distinctive *Chasmistes brevirostris* neurocranium in Klamath Lake, shown to be part of a hybrid complex with *Deltistes* (Fig. 9i) and two species of *Catostomus* (Markle et al., 2005; Dowling et al. 2016). Figure 10 illustrates the Utah Lake problem, in which introgression has been most extreme, commensurate with industrial and agricultural pollution as well as distortion of seasonal instream flow in the lower Provo River and Utah Lake (Mock et al., 2006; Cole et al., 2008).

Principal Component Analyses.— Shape measures of jaw bones (Fig. 12a), lip and mouth morphology (Fig. 12b), and dorsal aspects of neurocrania (Fig. 10) discovered no bimodal groupings of specimens separating Lake Suckers vs. *Catostomus*. In each comparison, ordination of individual *Catostomus* and Lake Suckers by their shape measures reveals no clusters, but individuals scattered by the uncorrelated mixtures of traits among them. The Principal Component Analysis of neurocranial measurements in Fig. 10 shows the distinctiveness of *Catostomus tahoensis* on the left, the fossil *Chasmistes spatulifer* at the top, and *Chasmistes cujus* on the right, with all of the hybrid forms scattered in the center.

Molecular Comparisons.— Levels of sequence divergence were calculated for each sympatric pair of *Catostomus* x *Chasmistes* species. The mtDNA of Palisades suckers differs by 0.007-0.011 from *C. ardens* of the Bear River drainage (Table 5, 6; Mock et al., 2006), similar to levels of divergence among Palisades Reservoir suckers (0.004-0.008). Utah Lake *Ch. liorus* and *C. ardens* differ by 0.007 (Table 5, 6). Jackson Lake *Ch. muriei* and *C. ardens* are similarly close (Cole, 2014). By comparison, *Catostomus tahoensis* of the Lahontan drainage differs from the Bonneville-Snake River group of *Catostomus* and *Chasmistes* by 0.044-0.049. In Pyramid Lake, *C. tahoensis* and *Ch. cujus* differ by a large molecular distance of 0.132, while *Ch. cujus* differs from the geographically proximate *C. fumeiventris* of Owens Valley, California, by much less, 0.048 (Table 5, 6). In Upper Klamath Lake, *Ch. brevirostris* differs from *C. snyderi* by 0.002–0.003 and from sympatric *C. rimiculus* by 0.010, but from the more remote population of *C. rimiculus* from the Rogue River by 0.022. *Deltistes luxatus* differs from *Ch. brevirostris, C. snyderi* and sympatric *C. rimiculus* by 0.011-0.016 (Table 5, 6; and see Dowling et al., 2016). In the Colorado River, *Xyrauchen texanus* differs from *C. insignis* by 0.056, and from *C. latipinnis* by 0.067 (Table 5, 6).

Age Calibration.— The age of the oldest fossil (from Pickett Creek) from the 'large-scale' *Catostomus* clade, excluding *C. catostomus,* is estimated to be 9.5 Ma (data from Carpenter and Smith, this volume). The earliest *Chasmistes*

Table 5.— Genetic distance estimates among catostomid taxa. See Methods.

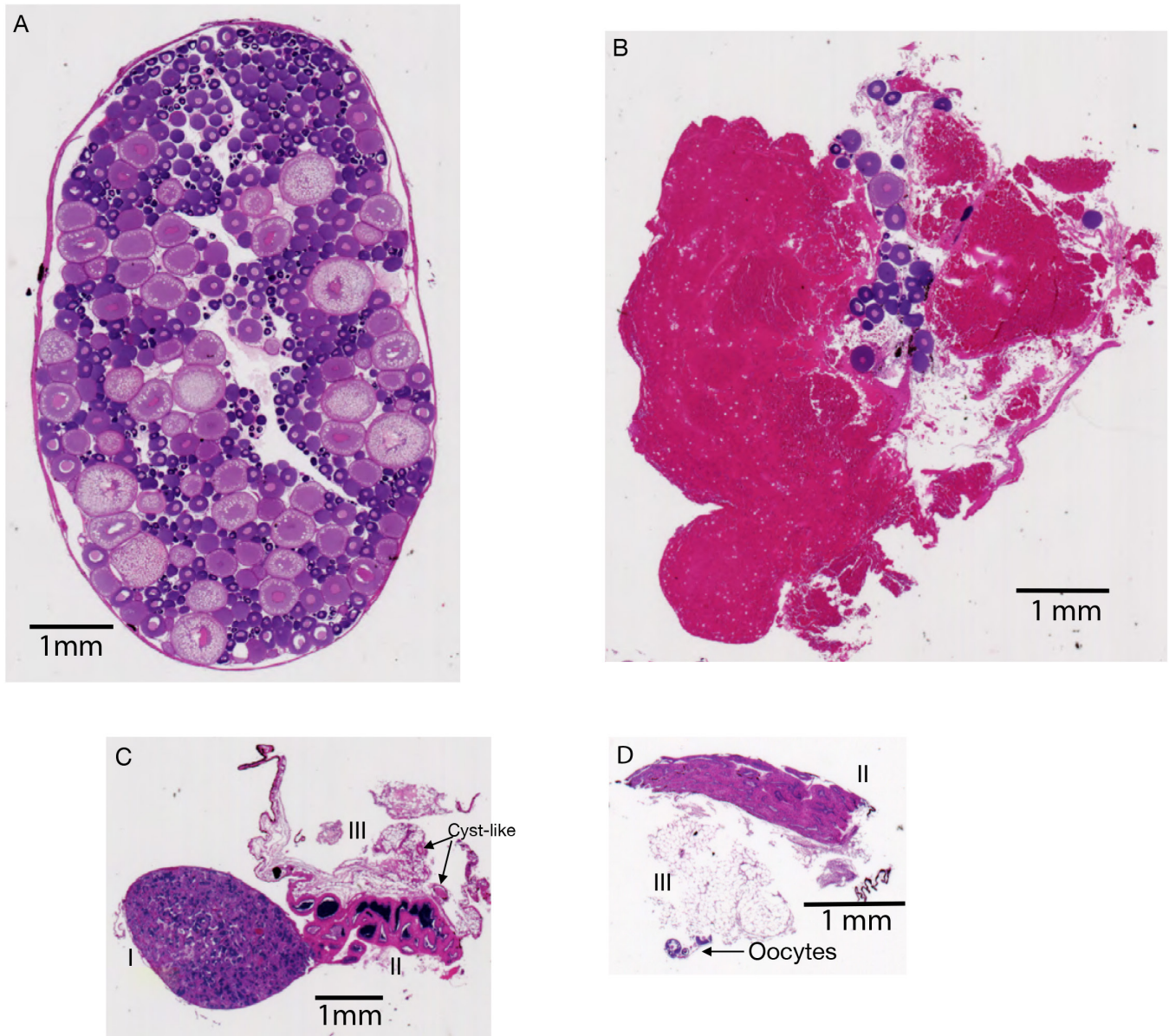|	C.ardens.bear.70212	C.ardens.utah.70154	C.catostomus.minncat1	C.commersoni.estcc1	C.fumieventris.fum1.OwensR	C.insignis.ecci1	C.latipinnis.grfm1	C.macrocheilus.paloose1	C.macrocheilus.279.Umpqua	C.rimiculus.rim266.133	C.rimiculus.rim149.L36	C.snyderi.br26.F3	C.snyderi.sny121.B11	C.tahoensis.tah2.Humboldt	c.wallcanyon.9081	C.warnerensis.war1	Ch.brevirostris.br4.B13	Ch.brevirostris.kt01.228.B50	Ch.cujus.cui1.PyramidL	Ch.liorus.70001	D.luxatus.lux304.132	D.luxatus.dl1.K	X.texanus.br254	L5919_LA	U51243_LA	X1020_LA
C.ardens.bear.70212	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.ardens.utah.70154	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.catostomus.minncat1	0.14	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.commersoni.estcc1	0.08	0.08	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.fumieventris.fum1.OwensR	0.12	0.13	0.19	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.insignis.ecci1	0.09	0.09	0.15	0.10	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.latipinnis.grfm1	0.09	0.09	0.15	0.09	0.14	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.macrocheilus.paloose1	0.03	0.03	0.14	0.08	0.12	0.08	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.macrocheilus.279.Umpqua	0.02	0.03	0.14	0.08	0.13	0.09	0.09	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.rimiculus.rim266.133	0.11	0.12	0.17	0.11	0.14	0.13	0.13	0.11	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.rimiculus.rim149.L36	0.11	0.11	0.17	0.11	0.12	0.13	0.12	0.11	0.11	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.snyderi.br26.F3	0.11	0.12	0.17	0.11	0.13	0.13	0.12	0.11	0.11	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.snyderi.sny121.B11	0.11	0.11	0.17	0.11	0.13	0.13	0.12	0.11	0.11	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.tahoensis.tah2.Humboldt	0.05	0.05	0.16	0.10	0.13	0.11	0.10	0.05	0.05	0.13	0.13	0.13	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
c.wallcanyon.9081	0.13	0.13	0.19	0.12	0.09	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
C.warnerensis.war1	0.12	0.13	0.18	0.12	0.08	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Ch.brevirostris.br4.B13	0.11	0.12	0.17	0.11	0.13	0.13	0.12	0.11	0.11	0.01	0.02	0.00	0.00	0.13	0.13	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Ch.brevirostris.kt01.228.B50	0.11	0.12	0.17	0.11	0.13	0.13	0.12	0.11	0.11	0.01	0.02	0.00	0.00	0.13	0.13	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Ch.cujus.cui1.PyramidL	0.12	0.12	0.18	0.12	0.05	0.13	0.13	0.11	0.12	0.12	0.12	0.12	0.12	0.13	0.08	0.07	0.12	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Ch.liorus.70001	0.03	0.01	0.13	0.08	0.12	0.08	0.09	0.03	0.03	0.02	0.02	0.02	0.02	0.05	0.13	0.13	0.12	0.12	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0
D.luxatus.lux304.132	0.11	0.12	0.17	0.11	0.13	0.13	0.13	0.11	0.11	0.02	0.02	0.01	0.01	0.13	0.12	0.12	0.01	0.01	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0
D.luxatus.dl1.K	0.11	0.12	0.17	0.11	0.13	0.13	0.13	0.11	0.11	0.02	0.02	0.02	0.02	0.14	0.13	0.12	0.02	0.02	0.12	0.01	0.00	0.00	0.00	0.00	0.00	0
X.texanus.br254	0.09	0.09	0.16	0.11	0.16	0.06	0.07	0.09	0.09	0.14	0.14	0.14	0.14	0.11	0.16	0.16	0.14	0.14	0.14	0.09	0.14	0.14	0.00	0.00	0.00	0
Palisades LS 5919	0.01	0.03	0.14	0.08	0.12	0.09	0.08	0.03	0.02	0.11	0.10	0.11	0.11	0.05	0.12	0.12	0.11	0.10	0.11	0.03	0.11	0.11	0.09	0.00	0.00	0
Palisades UT 1289	0.01	0.03	0.14	0.08	0.12	0.09	0.09	0.03	0.02	0.11	0.11	0.11	0.11	0.04	0.12	0.12	0.11	0.11	0.11	0.03	0.11	0.11	0.09	0.00	0.00	0
Palisades hybrid 1020	0.01	0.03	0.14	0.08	0.12	0.09	0.09	0.03	0.03	0.11	0.11	0.11	0.11	0.05	0.13	0.12	0.11	0.11	0.12	0.03	0.11	0.11	0.09	0.01	0.01	0

Figure 13.— Hematoxylin and eosin stained catostomid gonad sections. (A) All stages of ovarian growth are present in the healthy ovary section from gonad 19. (B) Relatively few healthy oocytes are visible amid widespread atresia in gonad 17. (C) In addition to testis tissue (I), there is possible ovotestis (II) and adipose (III) with cyst-like structures in gonad 7. (D) In addition to apparent ovotestis (II), gonad 25 contains three oocytes within adipose tissue (III).

are from the latest Miocene of eastern California and eastern Oregon (Miller and Smith, 1981; Smith et al., 1982). The number of 1 m.y. fossil horizons containing *Catostomus* is nine (Carpenter and Smith, this volume), for which the correction factor, alpha, is 0.09 for confidence interval ($C_{.50}$ from Marshall, 1990). The $C_{.50}$ is used to find the center of the age estimate distribution; $C_{.95}$ is used to find the 95% confidence interval around the estimate. The overall correction

is 9.6 x 0.09= 0.9 m.y. Therefore, the corrected age estimate of the split of the large-scale clade and *C. catostomus* is (9.5 + 0.9 m.y.) = 10.4 Ma. The average of pairwise estimates of sequence divergence for 15 members of the 'large-scale clade' from *Catostomus catostomus* is 10.9% (Table 5), which when divided by 10.4 Ma, gives a pairwise divergence rate of 10.9% / 10.4 m.y. = 1.05% per m.y. This fossil-based calibration and pairwise estimates of mtDNA sequence divergence were used
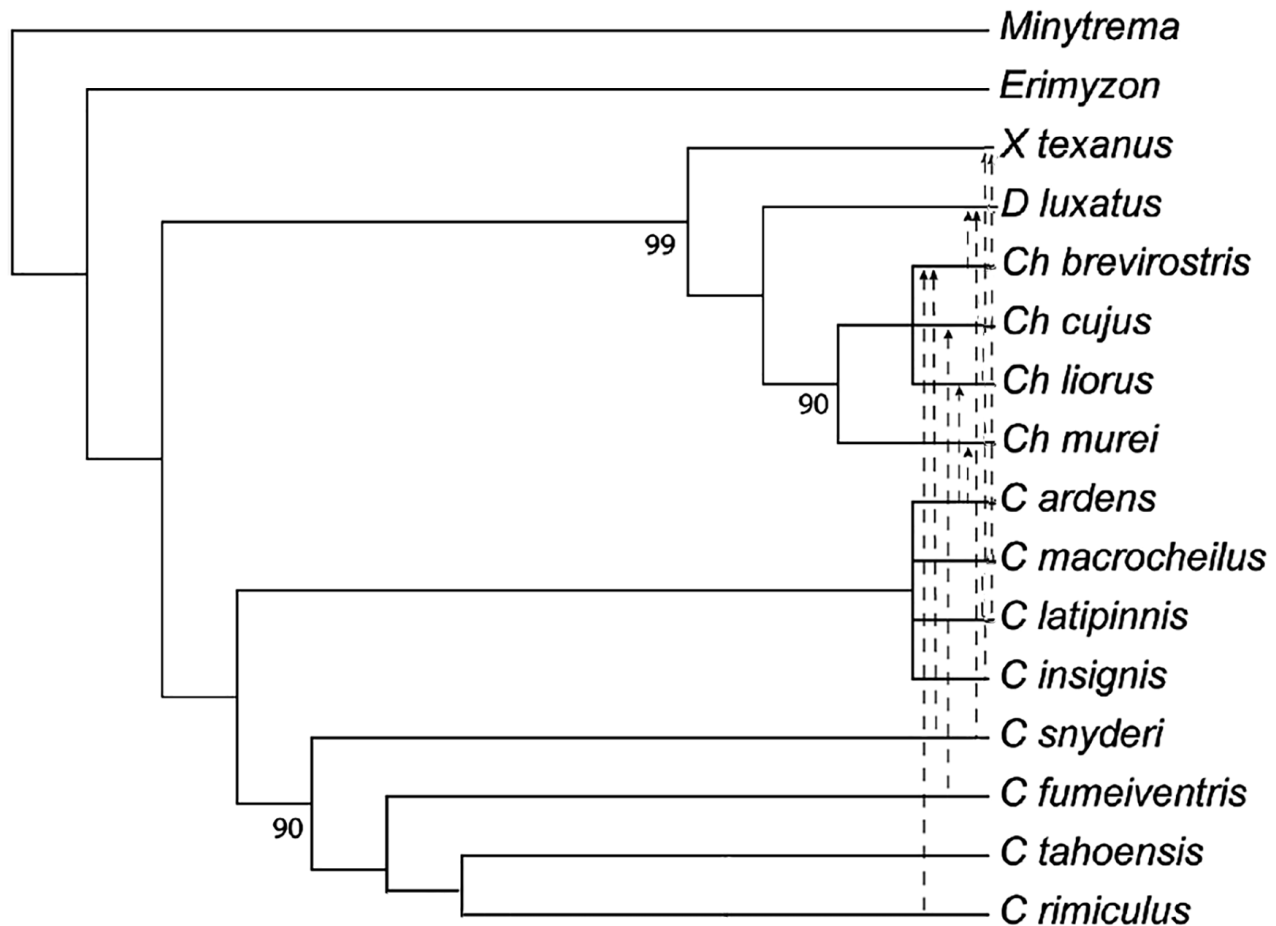
Figure 14.— Morphological tree showing branching relationships of *Catostomus* and Lake Suckers, *Chasmistes*, *Deltistes*, and *Xyrauchen* (after Smith, 1992a). Dashed arrows show direction of mtDNA replacements between sympatric or formerly sympatric species.

to obtain divergence time estimates for sympatric pairs of lake and river suckers (Table 6) for which there are fossils. These time estimates range between 0.24 Ma for *Ch. brevirostris – C. snyderi* and 5.9 Ma (for *X. texanus – C. insignis*).

Histology of Gonads.— Gross anatomy of gonads of 29 suckers from Palisades reservoir at the time of dissection included 14 females with seemingly normal ovaries and 15 males with abnormal testes (Table 7). The texture of the presumed testes was not firm and homogeneous like expected but appeared lacy, stringy, or loosely aggregated. Examination of stained slides confirmed 14 normal ovaries (Table 7, numbers 4, 5, 8-11, 14-16, 18-20, 22, 24) containing multiple stages of ovarian follicles as expected for fishes that produce multiple-clutches and are group-synchronous spawners. These females would have been capable of ovulating viable eggs. Females 11 and 14-16 showed varying signs of atresia but mostly contained healthy oocytes. Female 24 was thought to

be a male in the field, but stained sections revealed a healthy ovary in which the most advanced clutch of oocytes was in the lipidic stage (Table 7).

Male number 7 contained testis and abnormal tissue (Table 7, Fig. 13C). Male number 17 (Table 7, Fig. 13B) had ovotestes; gonad 17 was seriously abnormal—most of the tissue is atretic with a few developing oocytes (Fig. 13B). Male number 21 contained some lacy connective tissue, possibly from an atretic, malformed, ovotestis. Male 23 contained testis, possible ovotestis, and unidentified tissue. Males 25-30, thought to be males in the field, are abnormal, with tissue containing some oocytes, and some possible ovotestis. The sex ratio of normal fishes was significantly biased toward females; no normal males were observed. Males were generally smaller than females. A sample of 73 males, 85-365 mm in Standard Length, averaged 268 mm in Standard Length; a sample of 31 females, 133-444 mm in Standard Length, averaged 305

Table 6.— Estimated time of last gene exchange among introgressed pairs of taxa, in millions of years. The calibration ratio for these suckers, 1.05 percent sequence divergence per million years, is based on the age and percent sequence divergence of the basal split in *Catostomus*, see text. The sequence divergence between sympatric species times the calibration ratio gives the estimated time since last gene transfer.

Species pair	Calibration (% / Ma)	Sequence divergence (%)	Fossil age uncorrected	Gene contact time, m.y.
Ch. brevirostris x *C. snyderi*	1.05	0.25	no fossils	0.3
Ch. liorus x *C. ardens*	1.05	0.7	11 Ka (*liorus*, *ardens*)	0.7
Ch. muriei x *C. ardens*	1.05	0.9	5 Ma (*muriei*)	0.9
D. luxatus x *C. snyderi*	1.05	1.4	7 Ma (*Deltistes*)	1.5
Ch. cujus x *C. fumieventris*	1.05	4.8	6 Ma (*Chasmistes*)	5.0
Xyrauchen x *C. insignis*	1.05	5.6	5 Ma (*Xyrauchen*)	5.9

mm in Standard Length. Slides were compared with published examples of *Catostomus commersoni* gonads from Colorado (Vajda et al., 2008; Norris et al., 2018).

DISCUSSION

Chasmistes and *Deltistes* are genera of Lake Suckers adapted to large, productive lakes and their tributaries in Western U.S., especially the Pliocene rift lake on the Western Snake River Plain. Members of the Lake Sucker clade, including *Xyrauchen*, originated as large-lake species as inferred from their lacustrine sister groups (Smith, 1992a, 1992b), fossil record, and success of modern populations in reservoirs (Fig. 1; Dowling et al., 2005, 2014; Smith, 1975; Smith et al., 1982; Hoetker and Gobelet, 1999). But because lake silt is not as conducive to survival of fish eggs and larvae as river gravel, Lake Suckers typically spawn in tributary rivers in the Spring, sometimes near resident and migratory *Catostomus,* which usually inhabit and spawn in rivers. Lakes with *Chasmistes* and *Deltistes* are large, formerly supporting enormous numbers of suckers (Madsen in Jordan and Gilbert, 1882; Smith et al., 1982), including *Catostomus* and hybrids (Cope and Yarrow, 1876; Jordan, 1891; Tanner, 1936; Miller and Smith, 1981); spawning runs included tens of thousands of individuals.

Chasmistes evolved from about 7 Ma to present in the Walker Lane tectonic trough (16 Ma to present) at the shear zone between the Sierra Nevada and western Great Basin (Fig. 1; Faulds et al., 2005). Pyramid Lake, Nevada, and Upper Klamath Lake, Oregon, are associated with this zone. *Chasmistes* and *Deltistes* reached their zenith in the Glenns Ferry rift lake on the Western Snake River Plain, 4.5 to 1.8 Ma (Fig. 1; Smith et al., 1982; Carpenter and Smith, this volume). *Xyrauchen* has been endemic to Imperial Valley and the Colorado River since the late Miocene (Hoetker and Gobelet, 1999). Earlier, the Colorado Plateau was lower and contained large lakes (Hunt, 1969). The upper Colorado River connected to the lower Colorado River Imperial Valley in the Pliocene.

Hybridization between Lake Suckers and *Catostomus* occurred in the Pliocene, Pleistocene, Holocene, and recent according to fossil and molecular evidence.

Significance of mtDNA and Morphological Discordance.— Similarity of neutral molecular markers (AFLPs, mtDNA, microsatellites) in suckers of Jackson Lake and Utah Lake (reviewed in Cole 2014) is the basis of a hypothesis that *Chasmistes* is a recent local derivative of geographically proximate *Catostomus*, independently evolved in Utah and Jackson lakes. By implication, other Lake suckers are subject to this same hypothetical phenomenon (Chen and Mayden, 2012; Cole, 2014; Harris et al., 2014). Two hypotheses have been offered to explain the discordance between patterns of morphological and mtDNA variation among Lake and river suckers: *introgressive hybridization* and *responses to lentic conditions causing recent evolution of planktonic ecomorphs*. The introgression hypothesis (Miller and Smith, 1981) predicts (1) the observed evidence of a clade including *Chasmistes, Deltistes,* and *Xyrauchen*, supported by fossil and modern synapomorphies that diagnose the Lake sucker lineage*;* (2) a fossil record of morphologically divergent *Catostomus*, *Chasmistes, Deltistes*, and *Xyrauchen* demonstrating Mio-Pliocene apomorphy-diagnosed lineages, followed by Pleistocene and modern hybridization and genetic breakdown with loss of apomorphies of some modern Lake Suckers; and (3) evidence for current hybridization, backcrossing, and introgression between *Catostomus* species and their sympatric counterparts among Lake Suckers (Fig. 11). The benthic-limnetic ecomorph hypothesis (e.g., Cole, 2014) predicts (1) lack of traits diagnostic for a Lake Sucker clade; (2) no fossil record of divergent Lake Sucker genera and species; (3) recent, not ancient, minimal genetic divergence of *Chasmistes* from genomes of sympatric *Catostomus* species; (4) no evidence of hybrids, hybrid incompatibility, sterility or other genetic anomalies indicative of mismatched parental genomes; and (5) origin of limnetic Lake Suckers from *Catostomus* genomes*,* regulated by genes responding to environmental cues, and maintained by ecological selection, as in benthic/limnetic sticklebacks.

Table 7.— Summary of histological features of gonads of Palisades Reservoir hybrid suckers.

1. **Male,** 347 mm. Testis and ovotestis.

2. **Male,** 402 mm. Testis and ovotestis.

3. **Male**, 310 mm. Testis and ovotestis.

4. **Female**, 324 mm. Healthy ovary section from a multiple clutch, group-synchronous spawner. Nearly all stages of ovarian follicle development, from primary growth to late vitellogenic clearly visible. This female would have ovulated viable eggs.

5. **Female**, 298 mm. Healthy ovary. The dominant oocyte stage is primary growth, with a number of early- and mid-secondary growth oocytes visible. There are a few early vitellogenic oocytes as well. This female would have ovulated viable eggs.

6. **Male**, 389 mm. In addition to testicular tissue there is possible ovotestis and adipose tissue with cyst-like structures.

7. **Male**, 305 mm. In addition to testicular tissue (Fig. 13 C) there is possible ovotestis and adipose tissue with cyst-like structures.

8. **Female**, 344 mm. Nearly all stages of oocyte development, from primary growth to late vitellogenic stages clearly visible. This female would have ovulated viable eggs.

9. **Female**, 390 mm. Nearly all stages of oocyte development, from primary growth to late vitellogenic stages clearly visible. This female would have ovulated viable eggs.

10. **Female**, 403 mm. Nearly all stages of oocyte development, from primary growth to late vitellogenic stages clearly visible. This female would have ovulated viable eggs.

11. **Female**, 393 mm. Nearly all stages of oocyte development, from primary growth to late vitellogenic stages clearly visible. Despite the presence of some atretic oocytes, this female would have ovulated viable eggs.

12. **Female,** no slide.

13. **Female**, 308 mm. Nearly all stages of oocyte development are visible in this ovary and despite the presence of some atretic oocytes, this female would have ovulated viable eggs.

14. **Female**, 400 mm. This healthy ovary contains nearly all stages of oocyte development, from primary growth to late vitellogenic stages clearly visible. This female would have ovulated viable eggs.

15. **Female**, 410 mm. This ovary contains atretic oocytes; there are also healthy late vitellogenic oocytes visible. This female might have ovulated few viable eggs.

16. **Female**, 355. This healthy ovary contains all stages of ovarian follicle growth, from primary growth to the late vitellogenic stage. This female would have ovulated viable eggs.

17. **Male**, 433 mm. Most of this gonadal tissue is atretic with a few developing oocytes (Figure 13 B). This fish would have produced no spermatocytes and few oocytes.

18. **Female**, 433 mm. Nearly all stages of oocyte development present, from primary growth to late vitellogenic stages, clearly visible. This female would have ovulated viable eggs.

19. **Female**, 307 mm. Healthy ovary section. Nearly all stages of oocyte development, from primary growth to late vitellogenic oocytes, clearly visible (Fig. 13 A). This female would have ovulated viable eggs.

20. **Female**, 444 mm. Nearly all stages of oocyte development, from primary growth to late vitellogenic stages, clearly visible. This female would have ovulated viable eggs.

21. **Male**, 308 mm. Testis and unidentified tissue. The lacy tissue could be from an atretic, malformed, or immature ovary; the middle section may contain atretic oocytes. Darkly-stained tissue from testis.

22. **Female** 402 mm. Nearly all stages of oocyte development, from primary growth to late vitellogenic, clearly visible. This female would have ovulated viable eggs.

23. **Male**, 304mm. Testis and ovotestis.

24. **Female**, 220 mm. This is a health ovary likely from an immature fish. The most advanced clutch of oocytes within this ovary are in the lipidic stage.

25. **Male**, 187 mm. There are three oocytes visible in the section of gonad 25 (Fig. 13 D): the majority of the stained tissue is ovotestis.

26. **Male**, 295 mm. The majority of the stained tissue is normal testis with some ovotestis.

27. **Male**, 210 mm. Darkly-stained tissue from testis or ovotestis.

28. **Male**, 267 mm. Tissue near the oocytes could be from testis or ovotestis.

29. **Male**, 349 mm. Testis and atretic ovarian tissue.

30-1. **Male**, Testis tissue and atretic ovarian tissue.

The introgression hypothesis is supported by the long evolutionary history of morphologically diverged clade members *Chasmistes, Deltistes, Xyrauchen,* and *Catostomus,* documented in a detailed fossil record (Table 6; Fig. 13; Miller and Smith, 1967; Smith, 1975; Kimmel, 1975, 1982; Miller and Smith, 1981; Smith et al., 1982; Smith et al., 2000; Smith et al., 2002) and apparent male sterility in hybrids in Palisades Reservoir. Given the significant morphological divergence and long evolutionary history provided by the fossil record, the low, incongruent levels of mtDNA divergence most likely resulted from relatively recent introgression and asymmetric replacement of mtDNA. Introgression was possibly caused by Plio-Pleistocene climate fluctuations, including ancient droughts, and recently, human-induced water diversion, causing low in-stream flow leading to mixed spawning and introgression favoring river sucker mtDNA. Introgression of mtDNA is a common phenomenon in freshwater fishes (Ferris et al., 1983; Ferris, 1984; Wallis et al., 2017), and asymmetric introgressive replacement of mtDNA has been reported frequently, especially in western fishes (Gerber et al., 2001; Carson and Dowling, 2006), including suckers (Unmack et al., 2014; Dowling et al., 2016).

Contrasts of the fossil record with divergence time estimates from mtDNA support the introgression hypothesis. Calculated divergence times for mtDNA are small in three pairs (less than 1% sequence divergence implying recent introgressive hybridization or isolation less than 1 million years ago) between *Chasmistes* and sympatric *Catostomus* (Table 6), but the fossil record and cladistic estimates for the clade (Fig. 14) indicate that divergence of these genera probably occurred at least 7 million years ago. Disparate divergence times for *Chasmistes cujus, Deltistes,* and *Xyrauchen* and their *Catostomus* counterparts are commensurate with fossil ages and morphological cladistic estimates.

Chasmistes cujus, in relatively protected Pyramid Lake, obviously acquired its mtDNA from *Catostomus fumeiventris* (Fig. 14), which is endemic to Owens Valley, California, but was connected to drainages in Walker Lane in the Late Pleistocene or Pliocene (Reheis et al., 2002). *Chasmistes cujus* and *Xyrauchen texanus* exhibit large mtDNA divergence from sympatric *Catostomus,* congruent with evidence for persistence of morphological divergence, Miocene history, and inclusion in a monophyletic clade based on morphology. *Chasmistes cujus* and *X. texanus* showed less evidence for recent introgressive hybridization than other *Chasmistes* (Buth et al., 1987, 1992). *Xyrauchen* is sympatric with two species of river suckers, *Catostomus insignis* and *C. latipinnis*. The mtDNA from *Xyrauchen* is currently homogenous and shares a most recent common ancestry with *C. insignis*, indicating hybridization 5.9 Ma, presumably when drought and low in-stream flow led to mass spawning and introgression. In current times, *Xyrauchen* hybridizes with *C. latipinnis*, allowing for transfer of *C. latipinnis* mtDNA into *Xyrauchen* in the upper Colorado River basin. The presence of mtDNA of two species

of *Catostomus* in different populations of *Xyrauchen texanus* is not consistent with the hypothesis of ecophenotypic evolution from a parental *Catostomus*.

Sequence divergence estimates presented in the literature clearly document the most recent time that genes were exchanged, not the time of original cladistic branching. Evidence of fossil ages of *Chasmistes, Deltistes, and Xyrauchen* divergence from *Catostomus* occurred more than 5 m.y., earlier than ages of these lineages as inferred from small molecular divergences from *Catostomus* (Figs. 2, 14; Tables 5 and 6).

Lewontin (2002) suggested that the comparative study of variation is a useful way to gain insights into processes causing change. Because large samples of modern, non-introgressed *Chasmistes* do not exist, we compare a large sample of Pliocene jaw bones from *Chasmistes* in the large Glenns Ferry lake (4.5-1.8 Ma) to see the amount of variation. This allows a glimpse into history and circumstances unavailable from gene trees. Figure 8 shows a sample of mouth bones from a limited time at one of 35 localities, Crayfish Hill (Smith, 1975; Smith et al., 1982) that illustrate a moderate amount of population variation (ignoring breakage)—more variation than seen in average North American sucker populations, but less variation than seen in modern Lake Suckers (Fig. 7).

The pattern of variation seen in Figure 8 suggests phenotypic variation of the sort Lewontin described in his explanation of the interaction of genes, behavior, and environment expressed in the development of organisms that grew in different conditions. The key traits of the mouth in this genus, the anterior orientation of the premaxillary process of the maxilla (upper jaw) and the elongation of the anterior part of the lower jaw, are seen to be variable. We suspect that the unusual feeding adaptation of these midwater suckers for straining plankton (Willink, 2002) involved learned behaviors that influence the growth and hence the shape of these bones during life. There are rare indications of intermediates between *Ch. spatulifer* and *Catostomus*, referred to as *Chasmistes batrachops*. They do not satisfy predictions of the ecomorph hypothesis that invoke modification of the *Catostomus* genome after the individual fish encounters lake food and energy, because the jaw bones possess *Chasmistes* shapes at a very early age (Fig. 8r) and these shapes are not transmutable from shapes of *Catostomus* mouth bones. The gene-environment interactions seen in Figure 8 are interpreted as variations explained generally by Lewontin (2002) caused by individualized feeding behaviors (e.g., Wimberger, 1991; Yu and Albertson, 2017) among *Chasmistes* in a gigantic rift lake (Smith et al., 1982). Most suckers are benthic; *Chasmistes* are lentic. *Catostomus* genomes were not involved directly in their variation, except in hybrids.

Chromosomes and Genetics.— It is possible that survival and fertility of intergeneric sucker hybrids are facilitated by their allotetraploid chromosomes (Uyeno and Smith, 1972; Ferris, 1984). Hybrids are not variable or fertile until rare

backcross progeny survive and their progeny contribute to hypervariable populations (Fig. 11; Anderson, 1949; Hubbs et al., 1943; Hubbs and Hubbs, 1947; Hubbs and Miller, 1953; Hubbs 1955; Arnold, 2016). Hybrids between tetraploids (especially allotetraploids, Ferris, 1984; Zhou and Gui, 2017) have the potential to distort assortment and segregation. Formation of bivalents is usually a requirement for stable meiosis in allotetraploids because intergenomic recombination compromises the maintenance of the two parental chromosomal complements (Comai, 2005). Changes in regulatory networks and output pathways are thought to be deleterious, assuming the expression patterns of the parental species were optimized under selection. Polyploidy also changes the structural relationship between some cellular components and can alter mitosis and meiosis (see below). These effects might modify gene expression through reversible regulation or through epigenetic resetting (Comai, 2005). All of these distortions have the potential to increase morphological variability and cause conflicts in other cellular processes, possibly leading to inviability or sterility of disparate gene combinations. Testes of male hybrids from Palisades Reservoir are not well formed and often contain ova. A well-studied example of hybridization without introgression was documented by Bailey and Lagler (1938) among 1336 individuals of three species of sunfish in a small New York impoundment. The number of hybrids was 92, of which 80-96% were males, depending on the cross. Hybrids appeared to be sterile, based on abnormal gonads.

Hybrids vs. Ecomorphs.— Additional information comes from other species of *Catostomus* (specifically *C. catostomus* and *C. commersoni*), many of which occur in lakes across North America. The ecomorph hypothesis proposes that individuals of *Catostomus* exposed to lacustrine environments are triggered to develop from benthic into limnetic, plankton-straining suckers. This has never been observed, and evidence rejecting such transformations of *Catostomus* in lakes was documented by Geen (1958), Nelson (1968), and McElman and Balon (1980), among many others.

Identification of F_1, backcross, and F_2 hybrid individuals and populations of animals has often been controversial (Hubbs, 1955; Arnold, 2016). Natural hybridization is often denied (Hubbs 1955; Dowling and Secor, 1997; Arnold, 2016). Alternate hypotheses to explain intermediate morphotypes most commonly include proposed new species or ecomorphs. Although hybrid crosses and introgression (interspecific gene transfer past the first generation) have been demonstrated in thousands of plant and animal species (genetic experiments), deniers of commonness of hybrids often cite Mayr (1963) and Dobzhansky (1937), who negated the importance of introgression because they associated it with Darwin's belief in genetic continuity among species (Dowling and Secor, 1997, Argue and Dunham, 1999; Mallet, 2008).

The western American fish genera *Chasmistes*, *Deltistes*, and *Xyrauchen* include examples in which hybrids have been thoroughly analyzed and demonstrated, but individual cases often cause confusion in the field, because external traits are relatively uninformative, compared to trenchant internal branchial and bony differences. Unfortunately, internal differences are rarely studied. The features that make hybrid Lake Suckers obvious—mixed morphologies and one species with mtDNA and nuclear genes of the related form—sometimes opened the appealing possibility of new species or ecomorphs. But, these have been clarified by studies of hybrids using thousands of controlled crosses of cyprinids, salmonids, centrarchids, cichlids, moronids, goodeids, poeciliids, fundulids, and others, with experimental, analytical, and geographical methods for documenting F_1 and backcross hybrid inheritance (Hubbs, 1940; Hubbs and Miller, 1943; Hubbs and Hubbs, 1947; Hubbs et al., 1943; Hubbs, 1955; Hubbs and Miller, 1953; Neff and Smith, 1979; Bolnick et al., 2008). Robert Miller and his students carried investigations into hybrids in the fossil record (Miller and Smith, 1981; Smith, 1992b). The ecomorph hypothesis was tested by hatchery experiments on lean and fat forms of Lake Superior Lake Trout (Burnham-Curtis and Smith, 1994) and detailed hard food-soft food experiments on pharyngeal tooth molarization in Cuatro Cienegas cichlids by Jeff Taylor and Josh Trapani (Kornfield and Taylor, 1983; Trapani, 2003a, 2003b, 2004).

Cuatro Cienegas cichlids are a classic example of actual ecomorphs. These were thought by Minckley and Miller to comprise two to four species that differed in pharyngeal teeth and body shape (Taylor and Minckley, 1966; Minckley, 1969; Deacon and Minckley, 1974; LaBounty, 1974). Sage and Selander (1975), however, demonstrated that there are only two morphotypes among variants of Cuatro Cienegas cichlids, and because these possessed no genetic differences (see also Kornfield and Koehn, 1975; Kornfield et al., 1982), they concluded that only one species was present and that it contained two distinct tooth morphs, papilliform and molariform. Trapani (2003a, b) found no consistent differences in body shapes apart from pharyngeal teeth and the bones of the skull holding pharyngeal bones. Trapani (2004) analyzed six broods, wild-caught at Cuatro Cienegas, split experimentally, and fed either hard or soft food. Broods were not homogeneous. Genetic influence was demonstrated when the brood of origin influenced morphotype more than soft vs. hard food. Ecomorphs were demonstrated because some genotypes responded to hard food (snails) by ontogenetically replacing papilliform teeth with enlarged molariform teeth; papilliform teeth remained in cichlids fed soft food. These experiments demonstrated the interaction of inheritance and responses to environmental cues. None of these cases resembles the interaction of morphology and neutral genes seen in introgressed Lake Suckers.

The above systems have interesting implications for Lake suckers. Figures 10, 12a, 12b, and Table 4 suggest separate segregation of the study traits in hybrid crosses. The number of genetic differences involved in the morphological divergence is expected to be large, given ~7 million years

of evolution and the large differences in the neurocranium (Bookstein et al., 1985, fig. 4.7.10; Fig. 9). The inheritance of these morphological structures is apparently quantitative and polygenic. The genetic basis of the large differences appears to be broader than that for the trait differences involved in phenotypic modifications in cichlids (Huysseune and Sire, 1997) and benthic and limnetic sticklebacks (Glazer et al., 2015, Rennison et al., 2015, Erickson et al., 2016) because of the immense evolutionary time the divergence of Lake Suckers involved.

Inheritance of mtDNA.— Given the strong evidence supporting asymmetric introgression of mtDNA, it is important to consider possible causes. Clues to explanations of the uniquely-shared DNA between Lake Suckers and sympatric river suckers may come from among several phenomena: (1) All suckers are tetraploids. (2) Suckers normally spawn in rivers, where they may avoid hybridization in wetter climates by different spawning times and locations. (3) Hybridization is more likely during droughts when low water levels force the two species onto the same spawning beds at the same time (Hubbs, 1955). (4) Mitochondrial DNA is inherited only from the mother. (5) Hybrid male sex determination may be distorted by cyto-nuclear or similar conflicts leading to infertility (Orr and Turelli, 2001). (6) Some Lake Sucker hybrid males have ovotestes and are probably sterile. (7) Severe imbalance of species numbers or large fluctuations in species numbers affect incidence of hybridization (Hubbs, 1955).

There appears to be limited behavioral mate choice in these mass-spawning suckers (Reighard, 1920; Nelson, 1968). A hypothesis to explain one-way exchange of mtDNA postulates that affected hybrids contributed to future generations asymmetrically, because cyto-nuclear incompatibilities between mtDNA and nuclear DNA (Orr and Turelli, 2001; Turelli and Moyle, 2007) led to sterility or inviability of males.

Bolnick and Near (2005) demonstrated a strong negative regression between hybrid egg hatchability and divergence time in fishes. The fossil record shows that *Catostomus* diverged from Lake Suckers earlier than 7 Ma. (Smith et al., 1982; Carpenter and Smith, this volume). This provides a time perspective for application of five insights of Bolnick and Near (2005) and Bolnick et al. (2008):

1) Hybrid hatching inviability is the consequence of many small cyto-nuclear incompatibilities. This is demonstrated by the tight, steep, negative regression of hatching viability against fossil-calibrated divergence time that Bolnick and Near calculated. This observation contrasts with studies of *Drosophila,* which concluded that there were few large effects (but these studies were not measured against fossil-calibrated time).

2) Hybrid viability appeared not to drop below 100% until after 4-5 m.y. This lag time was interpreted as a 'snowball' effect of accrual of small incompatibilities or as compensating effects of heterosis. But this peculiarity

in the regression may be a consequence of incomplete early emergence of incompatibilities, which would be more numerous if measured later rather than at hatching of eggs.

3) Speciation ultimately depends on prezygotic mechanisms (time and place of spawning in Lake Suckers and *Catostomus*). This prezygotic conclusion was based on estimated time of speciation being shorter than time of evolution of inviability. But in suckers, the rate of accrual of sterility is underestimated by hatching time when compared to incompatibilities at maturity. Accrual of incompatibilities is part of speciation, but in these fishes it is ecological-context dependent, reversible, and not monotonic.

4) The amount of ecological divergence (measured as body size disparity) speeds up divergence. Adult *Chasmistes* and *Deltistes* are larger than *Catostomus*, but were not universally so in the Miocene and Pliocene (Smith et al., 1982).

5) Centrarchids retain the ability to hybridize longer than other groups; "Only Ictalurids are higher" (Bolnick and Near, 2005). Sucker genera exchange genes after 10-7 m.y. of divergence (Unmack et al., 2014; this paper), so they belong in this comparison. These groups may accrue postzygotic isolation slowly depending on distribution of sex-determining genes on chromosomes.

Since Lake Sucker mtDNA was eliminated and replaced by *Catostomus* mtDNA, we can work backward and investigate possible genetic mechanisms that acted on introgressing populations. The first mixed spawning of *Catostomus* x *Chasmistes* could have produced (1) 'pure' *Catostomus* males and females, (2) F$_1$ females (and males?) from the two kinds of reciprocal crosses, and (3) 'pure' *Chasmistes* males and females, depending on instability caused by polyploidy. F$_1$ hybrids are expected to be morphologically intermediate and uniform (Anderson, 1949; Hubbs, 1955), depending on deleterious interactions eliminating specific phenotypes. Viability, fertility, and sex ratios may not be equal if one sex (usually from a reciprocal cross and backcross; Orr and Turelli, 2001; Comai, 2005) are genetically disturbed and relatively infertile, possibly because of cyto-nuclear incompatibilities. In future generations, *Chasmistes* mtDNA would decline if, for example, hybrid males were sterile or progeny of male *Catostomus* and female *Chasmistes* were less fertile than the reciprocal cross. These considerations suggest a mechanisms by which Lake Sucker mtDNA could be lost over several generations of hybridization in which fertility of hybrid males was diminished by cytonuclear interactions.

Through subsequent generations, the decline of individuals with *Chasmistes* DNA could possibly lead to extinction (Rhymer and Simberloff, 2003). But Lake Suckers in large, productive lakes avoided extinction if those with branched

gill rakers and large subterminal mouths found unexploited plankton. According to this hypothesis, during drought periods, when mixed spawnings occur, reproduction will favor *Catostomus* and result in loss of Lake Sucker morphology and DNA, however, *Chasmistes* backcross products, with branched gill rakers, had a rich refuge in which to feed and flourish, but not breed. During wet climate cycles, fish with *Chasmistes* gill rakers could reproduce at different times and places and produce enough progeny with *Chasmistes* traits to thrive in the planktivorous niche in large lakes such as Pyramid, Upper Klamath, Utah, and Jackson lakes. The fossil record indicates that this occurred in ancient large lakes such as Mono and Owens, California; Ringold, Washington; and Glenns Ferry, Idaho and Oregon (Miller and Smith, 1981). Selection apparently reinforced co-adapted parts of the Lake Sucker phenotype, but introgressive hybridization during Pleistocene (and earlier) warm, dry cycles apparently resulted in elimination of Lake Sucker mtDNA.

Unique morphological adaptations of *Xyrauchen* remain unexplained, but Early Miocene lakes existed on the Colorado Plateau (Hunt, 1969), and in the Bidahochi region (Dallegge, 1999; Spencer et al., 2008). Imperial Valley, California, has been an important *Xyrauchen* refuge for at least 5 million years (Hoetker and Gobelet, 1999). It is instructive that replacement of mtDNA was asymmetrical in *Xyrauchen* hybrids with two species of *Catostomus*, and *Chasmistes* and *Deltistes* hybrids with two species of *Catostomus*. These complex crosses cannot be explained by the ecophenotype hypothesis.

Summary.— Evidence from the disparity and mixing of diagnostic traits, abnormalities and asymmetries, the fossil record, and the trend toward breakdown of reproductive isolation with climate change are all consistent with an upsurge in hybridization and loss of mtDNA in Lake Suckers in the past 136 years of environmental degradation in Utah Lake, Klamath Lake, Pyramid Lake, and Jackson Lake, and their tributaries. Lake Sucker mtDNA was universally replaced earlier by *Catostomus* mtDNA from sympatric or formerly sympatric species in *Xyrauchen, Deltistes,* and all *Chasmistes*, possibly enabled by cyto-nuclear incompatibilities and sterile male hybrids. Pyramid Lake was protected by the geologic separation between *Chasmistes cujus* and *Catostomus fumeiventris* and the modern protection by the Pyramid Lake Piute tribe, but *Catostomus* mtDNA had already replaced *Chasmistes* mtDNA.

The ecomorph hypothesis does not explain the pattern of hybrid mixtures in *Catostomus* and Lake Sucker traits in lakes or the failure of *Catostomus* to produce lentic ecomorphs outside of the Walker Lane, Snake River, Great Basin, and Colorado River region. The asymmetric pattern of mtDNA introgression is significant, requiring further examination to understand the evolution of mtDNA inheritance, sex determination, and losses of intrinsic and extrinsic reproductive isolation in this group.

The question concerning persistence of introgression in the face of its costs to fitness is still unanswered. Possible hypotheses to explore include the role of recombination in creating unfavorable environments for internal parasites (Maynard-Smith, 1978). Lake Suckers can live for 30 years or more (Scoppettone, 1988) offering internal parasites sufficient numbers of generations to evolve adaptive immune responses to their host individual. Hybrids benefit from recombination and defenses new to the parasites. But cases in which hybrid males are sterile complicate the cost-benefit ratio, reducing the ability of sexual reproduction, alone, to strengthen responses to parasites through recombination. Lake Suckers might provide helpful conditions for exploring the benefits of recombination to parasite defense.

ACKNOWLEDGEMENTS

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